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NATURE

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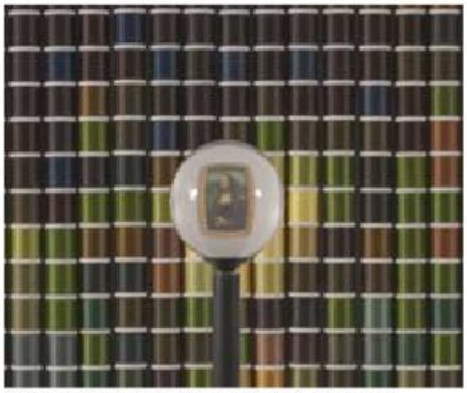

Smile: Mona Lisa in Manhattan. Books & Arts, p. **772**.

Joining the race to Antarctica, p. **752**.

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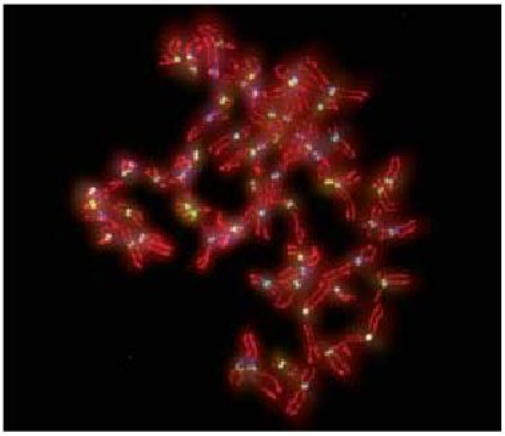

Cohesin seen in another light, pp. **796**, **777**.

ARTICLES

LETTERS

NATURE ONLINE

ADVANCE ONLINE PUBLICATION

PUBLISHED ON 10 FEBRUARY 2008

Resistance to therapy caused by intragenic deletion in *BRCA2*
S L Edwards, R Brough, C J Lord, R Natrajan, R Vatcheva, D A Levine, J Boyd, J S Reis-Filho & A Ashworth
doi:10.1038/nature06548

Secondary mutations as a mechanism of cisplatin resistance in *BRCA2*-mutated cancers
W Sakai, E M Swisher, B Y Karlan, M K Agarwal, J Higgins, C Friedman, E Villegas, C Jacquemont, D J Farrugia, F J Couch, N Urban & T Taniguchi
doi:10.1038/nature06633

PUBLISHED ON 13 FEBRUARY 2008

Transcriptional repression mediated by repositioning of genes to the nuclear lamina
K L Reddy, J M Zullo, E Bertolino & H Singh
doi:10.1038/nature06727

Translational control of the innate immune response through IRF-7
R Colina, M Costa-Mattioli, R J O Dowling, M Jaramillo, L-H Tai, C J Breitbach, Y Martineau, O Larsson, L Rong, Y V Svitkin, A P Makrigiannis, J C Bell & N Sonenberg
doi:10.1038/nature06730

BRIEF COMMUNICATIONS ARISING

PUBLISHED ON 14 FEBRUARY 2008

Arising from 'The human footprint in the carbon cycle of temperate and boreal forests' by F Magnani *et al.* *Nature* **447,** 848–850 (2007).

Nitrogen saturation and net ecosystem production
A De Schrijver, K Verheyen, J Mertens, J Staelens, K Wuyts & B Muys
doi:10.1038/nature06578

Ecologically implausible carbon response?
W de Vries, S Solberg, M Dobbertin, H Sterba, D Laubhahn, G J Reinds, G-J Nabuurs, P Gundersen & M A Sutton
doi:10.1038/nature06579

Reply: F Magnani *et al.* doi:10.1038/nature06580

THIS WEEK'S PODCAST

The cover this week shows the most primitive bat known. Its discovery resolves a long-standing mystery of bat evolution, as explained on the latest podcast. Also, how to go speed dating, microfibres that give you (or your nanodevices) energy, and the identification of an elusive supernova progenitor. Subscribe (for free) to the weekly programme or download single copies from iTunes or from:
www.nature.com/podcast

THIS ISSUE

RULES OF ATTRACTION In the name of research, Eli Finkel and Paul Eastwick watched hundreds of videos of singles taking part in 4-minute bouts of speed dating. They found, amongst other things, that the received wisdom that women go for money and men for beauty carries little weight, at least in initiating romantic liaisons. Matt Kaplan reports from the relationships front-line. [News Feature p. **760**; www.nature.com/podcast]

CULTURAL TRENDS Successful research on — and eventually therapy with — stem cells is dependent on reliable methods of cell culture. In this month's Technology Feature, Nathan Blow looks at the latest products

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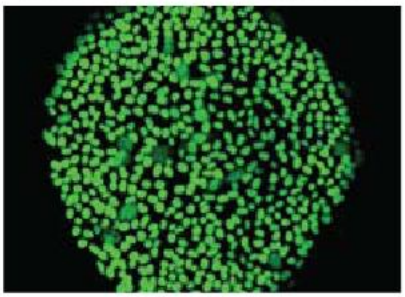


Cells on Matrigel, a basement membrane matrix.

from the major players in the market. The trend is towards a standard technology, or rather, to a few stable standard platforms from which researchers can develop systems tailored to their, or their cells', particular needs. [Technology Feature p. **855**]

ARCHITECTURAL FEATURES In *The Origins of Genome Architecture*, Michael Lynch has written what could become a classic text in evolutionary biology. Reviewer Axel Meyer rates it a 'must read' for every genome researcher, but cautions that Lynch's strong opinions — that largely non-adaptive forces shaped genomes — will not be accepted by all. [Books & Arts p. **771**]

REFORMED CHARACTERS The two names below look different, but both translate phonetically as Li Yan in English. That's an example of just one of the pitfalls facing Chinese researchers publishing in the lingua franca of science. But some

publishers are beginning to work out ways of overcoming the problems. Jane Qiu reports from Beijing. [News Feature p. **766**]

The Green River formation in Wyoming has produced many important fossils, including *Icaronycteris index*, which for over 40 years has been regarded as the oldest known bat. Its cranial features suggest that it could locate its insect prey by echolocation. This fuelled a spirited debate between proponents of the 'flight-first', 'echolocation-first' and 'tandem-development' hypotheses of bat evolution. New Green River bat fossils — including two near-complete skeletons, a cast of one of which is shown on the cover — looks to have settled the matter in favour of flight first. The new species is the most primitive bat known. It had fully developed wings and was clearly capable of powered flight, but the morphology of the ear region suggests that it could not echolocate, making it a possible intermediate link between bats and their non-flying, non-echolocating mammalian ancestors. Limb characteristics, including robust hind legs and retention of tiny claws on all of its elongate fingers, indicate that the new bat may have been an agile climber. [Letter p. **818**; News & Views p. **774**; www.nature.com/podcast]

AMERICAN MUSEUM OF NATURAL HISTORY

Nanomaterial: power dresser

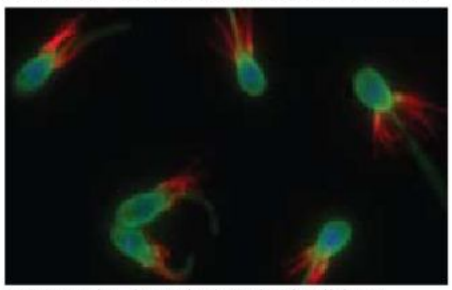
Nanodevices don't use much energy, and if the little they do need can be scavenged from vibrations associated with foot steps, heart beats, noises and air flow, a whole range of applications in personal electronics, sensing and defence technologies opens up. Energy gathering of that type requires a technology that works at low frequency range (below 10 Hz), ideally based on soft, flexible materials. A group working at Georgia Institute of Technology has now come up with a system that converts low-frequency vibration/friction energy into electricity using piezoelectric zinc oxide nanowires grown radially around textile fibres. By entangling two fibres and brushing their associated nanowires together, mechanical energy is converted into electricity via a coupled piezoelectric-semiconductor process. This work shows a potential method for creating fabrics which scavenge energy from light winds and body movement. [Letter p. **809**; www.nature.com/podcast]

Food webs: chaos reigns

Many mathematical models predict chaos in food webs, raising doubts over the extent to which we can predict future changes in species abundances as a result of climate change and habitat loss. This 'ecological' chaos has been demonstrated in simple model systems but not in real ecosystems, leading to suggestions that the real world may have a way of avoiding it. But now an extended (8 year) experimental study of a marine planktonic community isolated from the Baltic Sea has revealed 'naturally' chaotic population dynamics, implying that our ability to predict the long-term dynamics of real ecosystems may indeed be severely limited. [Letter p. **822**]

A key protozoan genome

The genome sequence of the marine choanoflagellate *Monosiga brevicollis* has now been determined. Choanoflagellates are a mainly sessile group of protozoa resembling the 'feeding cells' of sponges, and are considered to be the closest living unicellular relatives of multicellular animals. Comparison of the *M. brevicollis* sequence with metazoan genomes suggests that the last unicellular ancestor of animals had intron-rich genes, some encoding protein domains characteristically associated with cell adhesion and the extracellular matrix in animals. This organism is strictly unicellular, but other choanoflagellates form colonies and may provide clues as to the origin of cell signalling and other systems in early metazoans. [Article p. **783**]



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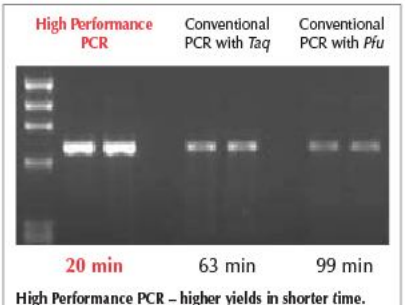

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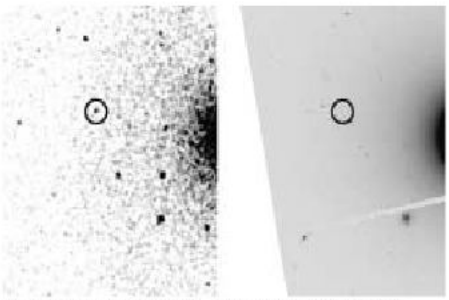
essential processes. Previous work identified a linear pathway in which five ethylene receptors converge on a single negative regulator, CTR1, and two key downstream components, EIN2 and EIN3. It remained a mystery how CTR1 regulates the downstream positive regulators. Now a previously unknown mitogen-activated protein kinase (MAPK) pathway involving MKK9 has been identified in *Arabidopsis*, acting positively to control EIN3-mediated transcription in ethylene signalling. Together, the antagonistic roles of CTR1 and MKK9 may determine ethylene-signalling specificity and quantitative responses via two MAPK phosphorylation sites with opposite effects on EIN3 stability. [Article p. **789**]

The other face of cohesin

A study of the distribution of cohesin binding sites in the human genome shows that they co-locate with sites for the transcription insulator protein CTCF. Cohesin is well known as a protein that acts as a 'chromosome glue', promoting sister chromatid cohesion during chromosome segregation. This new finding has uncovered a second and independent role for cohesin, facilitating CTCF function by acting as a transcription insulator. The discovery could be of medical relevance as a pointer to pathways that could be involved in 'cohesinopathies' such as Roberts syndrome and Cornelia de Lange syndrome. [Article p. **796**; News & Views p. **777**]

Primed to explode

Type Ia supernovae are exploding stars used to measure the expansion of the Universe. They are important to us as they are responsible for the production of most of the iron in the Universe. Exactly what object is exploding and why is unclear — though a white dwarf in a binary system is the likely candidate — and indirect methods of identifying progenitors have failed. Rasmus Voss and Gijs Nelemans now report the direct observation of a progenitor of a supernova that exploded on 5 November 2007. They unearthed an object in the exact position of supernova 2007on in images taken by the Chandra X-ray telescope four years before the explosion. Deep optical images from the archives show nothing in this position. This seems to favour the accretion model for this supernova, where a white dwarf accretes material from a companion star, rather than a merger of two white dwarfs. [Letter p. **802**; News & Views p. **775**; www.nature.com/podcast]



Then you see it (Chandra) — now you don't (Hubble).

Plant immunity pathways

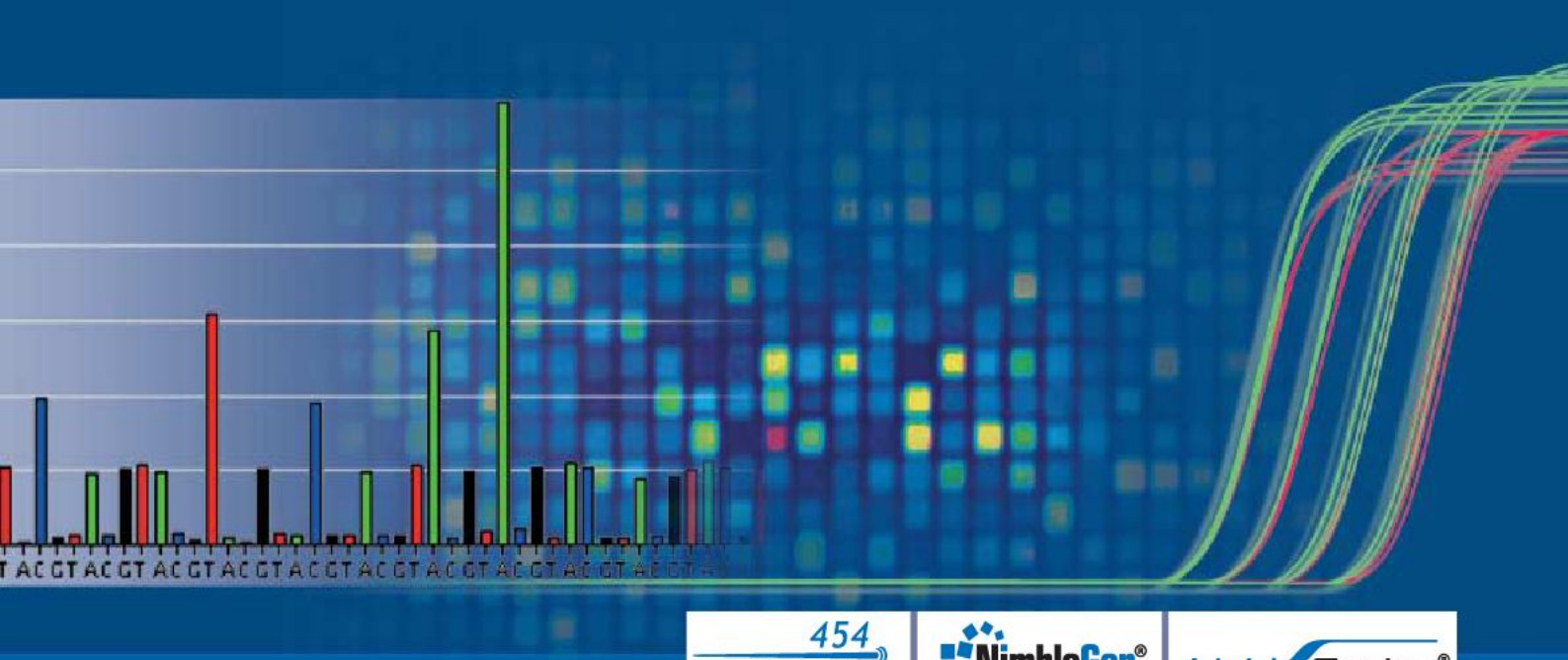
Plants successfully defend themselves against most fungal attacks, but the underlying mechanisms involved remain poorly understood. A study of *Arabidopsis* plants exposed to powdery mildew fungi has identified one component of the immune response as a ternary complex consisting a syntaxin protein called PEN1, the SNARE protein SNAP33 and two vesicle-associated membrane proteins, VAMP721 and VAMP722. Unexpectedly, the latter have a second functionally redundant role in a default secretory pathway. It is possible that an ancient secretory pathway involving these VAMP proteins was co-opted to form part of the secretory plant immune response. [Letter p. **835**]

Hypermutation on a leash

Somatic hypermutation, the mechanism by which activated B cells in the blood produce a diversity of immunoglobulin genes giving rise to high-affinity antibodies, plays a vital role in protecting the body from infection. Yet it also represents a major risk to genomic stability, with the potential to generate B-cell tumours if unchecked or wrongly directed. The somatic hypermutation reaction is initiated by activation induced deaminase (AID), and it is widely assumed that the risk of inappropriate hypermutation is averted by careful targeting of this enzyme. New work in mice suggests that this is not the case. Rather, AID deaminates a large fraction of the expressed genome, including numerous oncogenes linked to B-cell malignancies. Widespread mutation of the genome is averted in a surprising manner: by gene-specific, error-free DNA repair mediated by base excision and mismatch repair. [Letter p. **841**]

Three RNAPs is company

The Archaea were once thought of as the more primitive of the two prokaryotic lineages, but they are now considered to be more closely related to the eukaryotes (or Eukarya) than to the Bacteria. Determination of the crystal structure of archaeal RNA polymerase (RNAP) now allows a structural comparison of the transcription machinery between all three domains of life. The archaeal enzyme shows striking structural similarities to the eukaryotic equivalent, and should be a useful model system for the dissection of the molecular basis of eukaryote transcription. [Letter p. **851**]



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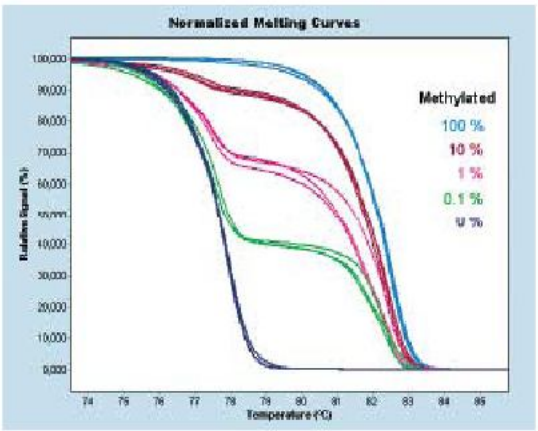

Analysis of tumor suppressor gene methylation with the LightCycler® 480 System. Mixtures of unmethylated and fully methylated genomic DNA were treated with bisulfite. A fragment of the MGMT tumor suppressor gene was then amplified and analyzed by high-resolution melting. This allowed semi-quantitative determination of the portion of methylated DNA contained in each case.

Data kindly provided by Tomasz K. Wojdacz and Alexander Dobrovic.

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Abstractions

SECOND AUTHOR

Ecological food webs are undeniably complex, but whether they are also chaotic has been a matter of debate for ecologists for more than 30 years. Chaotic systems are governed by physical laws so convoluted that long-term predictions of their behaviour are impossible. On page 822, Jef Huisman at the University of Amsterdam in the Netherlands and his colleagues present evidence that plankton food webs are chaotic. He tells *Nature* why ecology may never be able to make long-term predictions.

There are very few empirical examples of chaos in ecosystems. Why is that?

Because we need long-term studies to prove that ecosystems are chaotic. My co-author Reinhard Heerkloss monitored the abundance of plankton species and nutrient fluxes under controlled temperature and light conditions for 2,300 days. He said it looked as if the system behaved chaotically, but he didn't know how to prove it, so he gave me the data. Plankton have short generation times, so the data covered thousands of generations.

Did you find evidence of the 'butterfly effect'?

Yes. This classic example of chaos theory — that the flap of a butterfly's wings can cause tiny atmospheric changes that result in a tornado elsewhere — describes how slight differences in the initial conditions of a system can change subsequent interactions in that system, leading to large-scale variation. Initially, our plankton species behaved as predicted, but then the abundances of species slowly diverged until, after 15–30 days, we could no longer predict their behaviour. We found the same limit to predictability for all plankton species in the food web — strong evidence that interactions among species cause the ecosystem to behave chaotically.

Did your work resolve any other questions?

Yes. Plankton species compete for several limiting resources. Classical ecological theory suggests that only a handful of species should survive in one place. Yet sampling studies routinely find 60–100 species in a single millilitre of water. Our data show that chaotic fluctuations in species abundance, resulting from species interactions, may create temporal 'windows' for more species to invade and survive in the system.

Will ecology ever be a predictive science?

Predictions in ecology will be similar to those for the weather — short-term predictability is high, whereas long-term forecasts are all but impossible. That said, we need long-term studies to understand all the mechanisms at work when species interact with each other or the environment. ■

MAKING THE PAPER

Rasmus Voss

Archived X-ray data reveal the secrets of a supernova's formation.

A trip to see friends and give an informal talk about his graduate work turned out to be much more fruitful than Rasmus Voss had anticipated. The visit, to Radboud University in Nijmegen, the Netherlands, led to work that takes astronomers a step closer to establishing how one type of supernova forms.

At Radboud, Voss, now a fellow at the Max Planck Institute for Extraterrestrial Physics in Garching, Germany, spoke about his work analysing X-ray telescope data. Gijs Nelemans, a theoretical astronomer at Radboud, and the adviser of one of Voss's friends, attended the talk. He realized that with his knowledge of theoretical models and Voss's expertise, the two of them could tackle a model which suggested that the presence of X-ray signals could precede a type Ia supernova — an exploding star astronomers use to measure the expansion of the Universe.

At the time, two schools of thought prevailed about the origins of these supernovae. One theory was that supernovae result from the merging of two white dwarfs, which are small, dense stars. Such a merger would not be expected to be preceded by any X-ray data. The other possibility was that a white dwarf slowly accretes material from a companion star, producing X-ray signals in the process. If Voss and Nelemans found X-ray signals in positions at which type Ia supernovae later occurred, this would lend credence to the accretion model.

The origins of supernovae are difficult to study, because by the time researchers detect an explosion, the object that led to it has gone. So Voss and Nelemans decided to hit the archives in search of optical telescope data that showed supernova activity. Voss then trawled through earlier archival data from the Chandra X-ray Observatory, which can detect the most distant X-ray sources, to look for X-ray signals in locations at which supernovae had occurred. Their attempts, which focused on three supernovae that had occurred in 2002, 2004 and 2006, did not yield conclusive results.

The two decided to put the project on the back burner and wait for a new supernova to occur. "Not many of these events happen within a distance where they can actually be seen," says Voss. "So we basically sat for half a year. Then a nearby supernova exploded." But Voss didn't immediately scramble to the Chandra X-ray data archives, thinking that his chances of finding X-ray data to validate the accretion model were slim. "I had been working on another project," he says, "So I delayed looking at the data."

But when he did go back to the archives, he found the X-ray signatures that had eluded him on the other three supernovae. His reaction, he says, was a mixture of being "very embarrassed" for not immediately jumping on the data and "very excited" because the presence of X-ray signals preceding the supernova demonstrated the feasibility of their approach.

Their findings (see page 802), Voss says, do not exclude the merger model, because type Ia supernovae may occur by multiple means. But a likely outcome of the work is that more supernova specialists will start looking at X-ray data in their studies. And, as far as Voss is concerned, the next time he sees any supernova activity, he'll attack the X-ray data immediately. ■

FROM THE BLOGOSPHERE

A new web-based application aims to help scientists determine the journal most appropriate for publishing their results and select appropriate peer reviewers. The application, called Jane (journal/author name estimator), is described in the Nautilus post "What's in a Jane?" (http://blogs.nature.com/nautilus/2008/01/whats_in_a_jane.html).

Jane works by comparing sample text input by the aspiring author with that of published journal articles. At present, Jane has some teething troubles, as demonstrated by a trial run, but could an automatic selector ever be the best method of selecting the journal in which to publish one's results?

Suggestions and advice are readily available from scientists in the field, who hear about work at talks or read about it in a preprint. And journals provide author guidance on their websites about editorial scope, impact factor and so on. *Nature*, for example, is looking for novel results, not something similar to work that has just been published. It will be a sad day, according to Nautilus, when science journals publish articles selected for them by computer. ■

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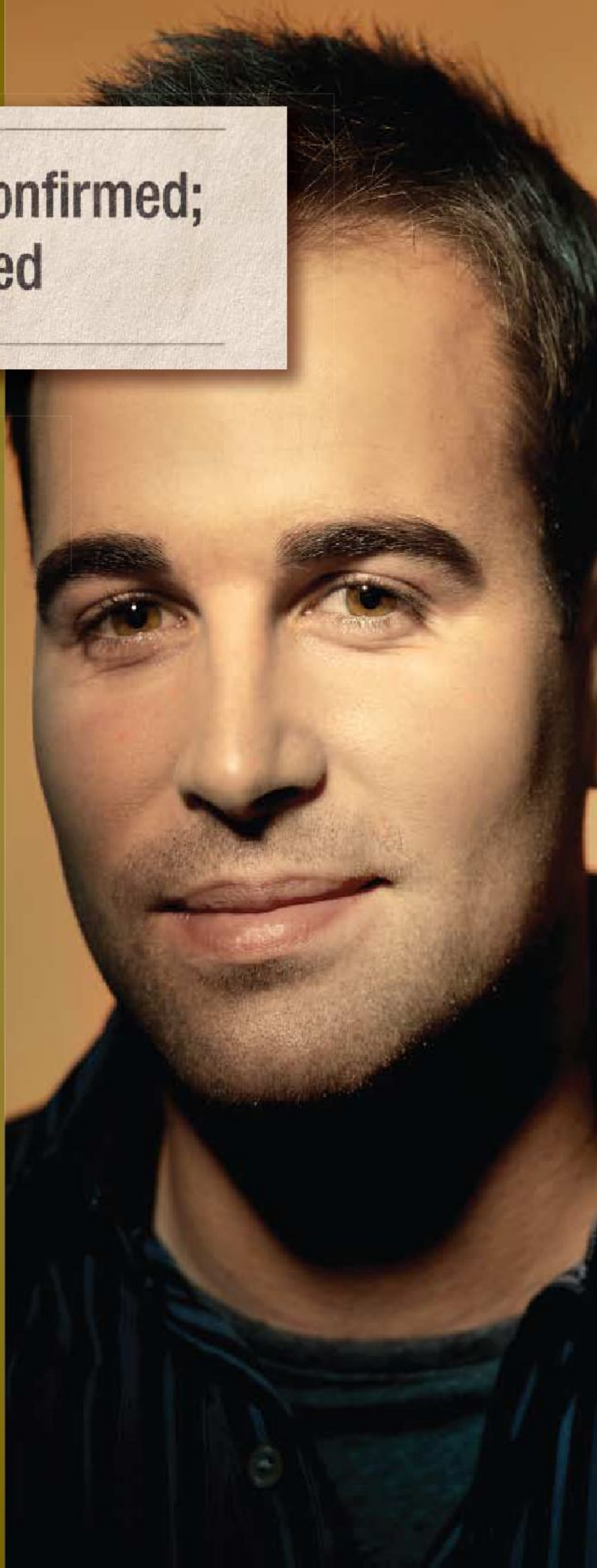
Protein interaction confirmed; pathway characterized



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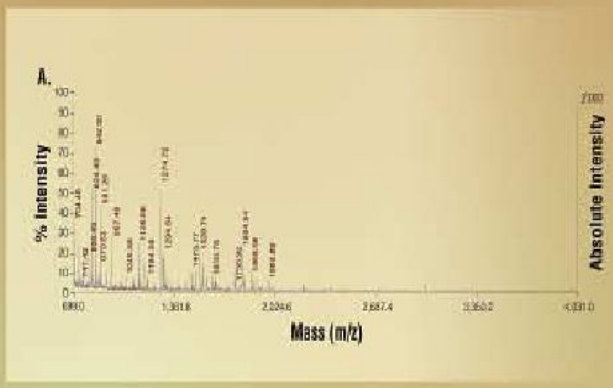
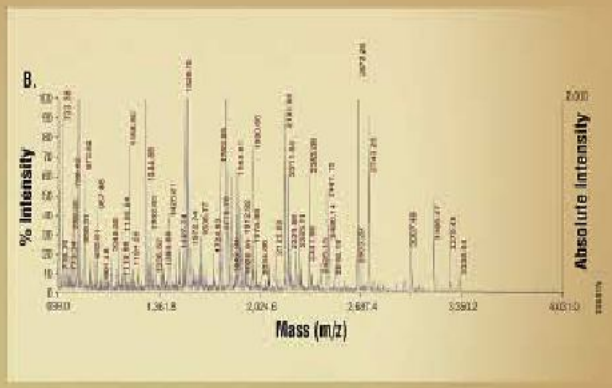


Panel A: Current standard: in-gel digestion of 47kDa protein band from mouse cytoplasmic protein extract using Trypsin Gold.



Panel B: More complete in-gel digestion of 47kDa protein band from mouse cytoplasmic protein extract using Trypsin Gold with ProteasMAX™ Surfactant.

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nature REVIEWS MOLECULAR CELL BIOLOGY

Free Poster – Targeting lipid signalling in disease

Lipids are important mediators in cancer, inflammation and in cardiovascular, neurodegenerative and metabolic diseases. A complex protein–lipid signalling network that comprises phosphoinositides, sphingolipids, steroids and other lipid-derived mediators has been uncovered in recent years.

This Poster provides a schematic overview of the protein–lipid signalling network and how this network can be exploited pharmacologically to attenuate proliferative, inflammatory and metabolic diseases.

The Poster accompanies a special Focus on Lipids (www.nature.com/nrm/focus/lipids), the content of which is freely available throughout February 2008.

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Vol 451 | Issue no. 7180 | 14 February 2008

Nuclear security undervalued

The world's only agency for assuring global standards and security in nuclear installations needs an upgrade. This cannot be done on the cheap.

Think of the International Atomic Energy Agency (IAEA) and images come to mind of intrepid inspectors using the latest James Bond-like techniques to ferret out would-be nuclear proliferators. The reality is considerably less powerful. In theory, for example, mass spectrometry of microparticles has revolutionized the ability of inspectors to detect even tiny amounts of highly enriched uranium. In practice, they not only face a significant backlog of samples, but have to make do with instruments that are often three decades old and have no available spare parts.

And that is assuming that the inspectors have actually found the samples. The agency cannot afford to purchase sufficient commercial satellite imagery, much less possess its own satellite system, so too often it learns of the construction of suspect nuclear facilities from the media, or from non-governmental organizations.

Without a major budget increase for the IAEA, the world will not make the most of a vital player in defending against some of the greatest dangers it now faces. As billions of dollars pour into the expansion of nuclear power, and as more nations seek to join the nuclear club, the IAEA's budget has been flat at around €280 million (US$406 million) for much of the past two decades, despite an increasing workload. This stagnation is the result of a deliberate policy of 'zero real growth' imposed in the late 1980s by the richer members of the IAEA's 144 member states, who wanted to force international agencies to reform and seek external funds.

The agency's role is most deficient in the realm of nuclear security. In 2002, it created a Nuclear Security Fund in the wake of 9/11 to boost its almost non-existent efforts to help secure nuclear materials worldwide, and to detect and prevent nuclear terrorism. But the fund depends on voluntary contributions from member states, and its current annual budget of €15 million is not only wholly inadequate, it also includes some 'in kind' contributions, and is peppered with national earmarks.

Politicians worldwide claim that they have made nuclear security a priority since 9/11, but they must now translate that promise into action: at least double the IAEA's budget, a ballpark figure already suggested by the agency's director-general, Mohamed ElBaradei. Such a doubling of an already small budget is surely the least that would be consistent with the world's expectations of the agency.

Furthermore, the IAEA's member states should make the Nuclear Security Fund a regular part of the agency's budget, paid for by dues, so that it can be properly planned and staffed. It needs the means to hire and train a sufficient number of experts on nuclear security, to provide the international community with a detailed picture of nuclear security worldwide, and to focus its efforts in areas, such as the former Soviet Union, where they are most needed. It also needs greater powers — at present the IAEA can comment on what it sees as insecure facilities or stockpiles, but has no powers to enforce tighter security measures and upgrades, as these remain national prerogatives.

"There is a greater need for nuclear operators worldwide to openly share security and information among themselves."

There is also a greater need for nuclear operators worldwide to openly share security and information among themselves. One model is the World Association of Nuclear Operators, a forum created after the Chernobyl accident to bring together all the players in nuclear power to swap notes and review best safety practices. The US Nuclear Threat Initiative has proposed creating the same sort of forum, a World Institute for Nuclear Security, to discuss security issues. The idea is a valuable one that merits support.

ElBaradei has launched a '20/20 review' to redefine the IAEA's role in a world of expanding nuclear power and new security threats. The IAEA needs to be able to use the best technologies available, including mathematical modelling of material flows in sensitive facilities, and remote sensors, to better monitor diversion of nuclear material. It needs better databases of the fingerprints of illicit material to establish its origins. That will also require a new breed of inspector, less oriented towards checking the quantities of nuclear materials going in and out of facilities, and more detective-like, more inclined to rock the boat, and above all, better trained in the latest high tech. None of that will come cheap. No one is asking that the IAEA be given a blank cheque, but properly funding our international nuclear-verification system seems to be a bargain insurance policy. ■

Genetics benefits at risk

A rogue senator needs to be bypassed.

Technology development guru George Church — aka the information exhibitionist — is playing a salutary social role with his Personal Genome Project. Church is in the process of gathering phenotypic data and sequencing portions of the genomes of ten volunteers, including himself (see page 763). He intends to study how the genes of these people — all but one of whom have revealed their identities — influence their phenotypes, and to make those data public. Church's point is simple: information, including genetic information, can and should be freely available.

Whether or not one agrees with him, society had better be ready to deal with the results of such research, which is occurring against a background of explosive growth in the availability of genetic information. Consider that, in the five-and-a-half months since *Nature* last opined about this topic (see *Nature* **448**, 969; 2007), the number of

diseases for which genetic tests are available to patients has grown by 8.4%, to 1,236.

This makes it all the more urgent that the US Congress enact the Genetic Information Nondiscrimination Act, which would protect people from being discriminated against by health insurers or employers on the basis of their genetic information. The entire scientific and medical community is adamantly supportive of this bill — as is the House, which passed it on a vote of 420:3 last April. In previous Congresses, the Senate has twice passed it unanimously. These are sure signs of an idea whose time has come, given that no less powerful a lobby than the US Chamber of Commerce had twisted arms to try to prevent its passage.

But for seven months now, one senator, Tom Coburn (Republican, Oklahoma), has used a procedural manoeuvre called a 'hold' to prevent the bill from coming to a vote in the Senate. Although Coburn, a practising obstetrician, voted for the bill in past, he is now refusing to budge, knowing full well that if he does lift his hold and allow a vote, this would all but guarantee the bill's signing into law. (President Bush, during a visit to the National Institutes of Health a year ago, urged Congress to pass the bill; his administration also put out a written statement of policy supporting it.)

Coburn has said he won't support the bill without a change that would drive a huge hole through its protections. In essence, he wants to insulate employers from ever being sued for a discriminatory act if an insurer is also culpable in the same situation. To put a human face on it, let's say that Employer X, who is a major client of Insurer Y, asks Insurer Y to make life miserable for Employee Z by, say, denying her coverage based on a genetic predilection for breast cancer in her family. If Coburn had his way, the insurer could be fined, but the employer would be immune from being sued for damages by Employee Z.

It is hard to believe that Coburn is bargaining in good faith, given that he has put holds on at least 86 other pieces of legislation. Nonetheless, if his intention is to protect businesses from an avalanche of frivolous litigation, there's precious little to suggest that this is a serious danger, given the virtual absence of similar lawsuits in the 34 states that have independently enacted laws forbidding genetic discrimination by employers.

There is a way past Coburn. Under Senate rules, Democratic Senate Majority Leader Harry Reid could force the bill to a floor vote by allowing 30 hours of debate on it first. That kind of time is a precious commodity in a highly preoccupied Senate. But it's hard to imagine a more worthy cause for which to make room, or a more important legacy for this Congress — and scientists should tell Reid as much.

Otherwise, the enormous research and clinical progress being made in the nascent era of personalized medicine will come crashing to a halt because people — despite the efforts of George Church — will remain rightly wary of taking genetic tests. ■

Down on the farms

Efforts by the Ministry of Defence to block offshore wind farms is at odds with Britain's long-term security.

When the UK government set out its energy policy in the spring of last year, it was heralded as one of the most ambitious programmes in Europe. The government pledged to reduce carbon dioxide emissions from 1990 levels by at least 26% in 2020 and by 60% in 2050.

Wind energy is poised to play a small but critical role in the government's green scheme. Wind power is among the most competitive of all renewable energy sources, and it will help the government to meet near-term goals — most notably, a 10% reliance on renewable energy by 2010. At present, there are 2.3 gigawatts of installed capacity in Britain, and current projects in planning could bring that number to 33 gigawatts by 2020.

That is, if developers can convince the Ministry of Defence (MoD) to let them build. The MoD has opposed wind farms for more than a decade, on and off, on the basis that they interfere with air-defence radars. Construction of turbines requires MoD approval during the planning stages, and last week the ministry announced that it is blocking four offshore wind farms planned for the North Sea. The farms, it claimed, would interfere with radar stations along the coast.

The MoD's current policies are at odds with those of other European nations that depend on wind energy. Austria, Germany and the Netherlands all require a review of wind farms built within a certain range of military installations, but none have the ministry's blanket policy demanding sign-off on all turbines within any radar line-of-sight. Given that air-defence radar must, by definition, cover the entire coastline, the rule effectively means that the MoD can block any offshore farm.

Its concerns do deserve consideration. The massive blades of wind turbines have an enormous radar cross-section that can make it more difficult to see behind them. That has led to fears that a low-flying aircraft or cruise missile might be able to use the farms as cover for a stealth attack. More critically, spinning blades interfere with a radar's Doppler system, creating false contacts and blinding operators to the area above the farm. Ongoing studies by the MoD and the US Department of Defense show that the overall effect depends both on the position of the farm and the type of radar used.

But there is no reason to think that the radar problem is insurmountable. Signal-processing hardware and software now in development should be able to remove a part of the radar 'clutter' from the area around wind farms. A simpler, though more costly, solution would be the strategic placement of a secondary radar that could either look behind the farms or above them. These solutions are not thought to be prohibitively expensive. Developers, eager to see their turbines turn on, have even expressed a willingness to share in the cost of some necessary upgrades.

The mission of the MoD is to defend the United Kingdom and its interests. In the modern world, the threat of climate change is as real as any military or terrorist threat, and energy supply is a critical aspect of national security.

The technical issues are real, but there is every reason to believe that they can be dealt with using technology that either already exists or can be developed in the near term. It is time for the ministry to end its war with wind-power developers and adopt a more cooperative spirit. ■

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RESEARCH HIGHLIGHTS

Kissing cousins

Science **319**, 813–816 (2008)

Close inbreeding is typically frowned on by society and geneticists alike. But research in Iceland has shown that pairing up with a third or fourth cousin may actually boost fertility.

Agnar Helgason and his colleagues at deCODE Genetics, a company in Reykjavik, reached this conclusion after analysing genealogical data for 160,811 couples born in a 165-year period starting from 1800. Icelanders remained remarkably equal during this time in both social and economic terms, so the authors assumed that these factors exerted little influence on marital unions.

For example, women born between 1925 and 1949 who married third cousins had, on average, almost one more child, and almost two more grandchildren, than couples who were eighth cousins or even more distantly related. First- or second-cousin marriages had just as many kids as third-cousin unions, but their children died younger and were less likely to reproduce.

D. TELEMANS/PANOS

SYSTEMS BIOLOGY

Critical condition

Proc. Natl Acad. Sci. USA **105**, 1897–1900 (2008)

The idea that life is a self-organized 'critical state' akin to those postulated for physical systems such as avalanches has long been mooted in complexity science. Ilya Shmulevich of the Institute for Systems Biology in Seattle, Washington, and his team have now found a signature of critical dynamics in the gene-expression pattern of immune cells called macrophages.

The team measured the information content of transcriptional activity in macrophages as the cells responded to various stimuli over time. Transcriptional states triggered by different stimuli walk a fine line, they found, becoming neither more nor less like one another as time progresses. This means that the corresponding changes in the cells' information content are poised on a knife edge between order and chaos.

The balance corresponds to a critical state, which the researchers think offers an ideal compromise between stability and adaptability in the cells' responses.

MATERIALS SCIENCE

In and out

Science **319**, 794–797 (2008)

A UK-based collaboration has found a crystal that when cooled contracts in one direction while expanding in another, changing its dimensions by ten times the amount of a typical crystal.

Andrew Goodwin at the University of Cambridge and his colleagues probed silver hexacyanocobaltate ($Ag_3[Co(CN)_6]$), a structure composed of long strings of colbalt, silver, nitrogen and carbon atoms arranged in a lattice. When the crystal cools, silver atoms are drawn together, contracting the compound in one direction. This causes cobalt atoms, which occur between silver atoms along the string, to be pushed apart, expanding the material in the other direction.

The crystal might one day be used to better control systems that experience large temperature swings, such as spacecraft instruments, the authors say.

ENVIRONMENTAL MODELLING

Surprise supply

Environ. Sci. Technol. **42**, 822–830 (2008)

It has long been supposed that cultivated croplands are the largest source of phosphorus, as well as nitrogen, in the Mississippi river. But the latest water-quality model developed by the US Geological Survey suggests otherwise.

Richard Alexander at the agency's headquarters in Reston, Virginia, and his colleagues think that animal manure on pasture and rangeland accounts for 37% of the phosphorus moving into the river. This then travels onwards into the shallow coastal waters of the Gulf of Mexico, where it contributes to a hypoxic 'dead zone' in summer. By comparison, corn and soya bean cultivation account for only 25% of phosphorus discharged into the Mississippi, but 52% of nitrogen pollution.

That the distribution of pollution sources is more diverse than was previously supposed suggests that managing runoff in the Midwest may be more complicated than thought.

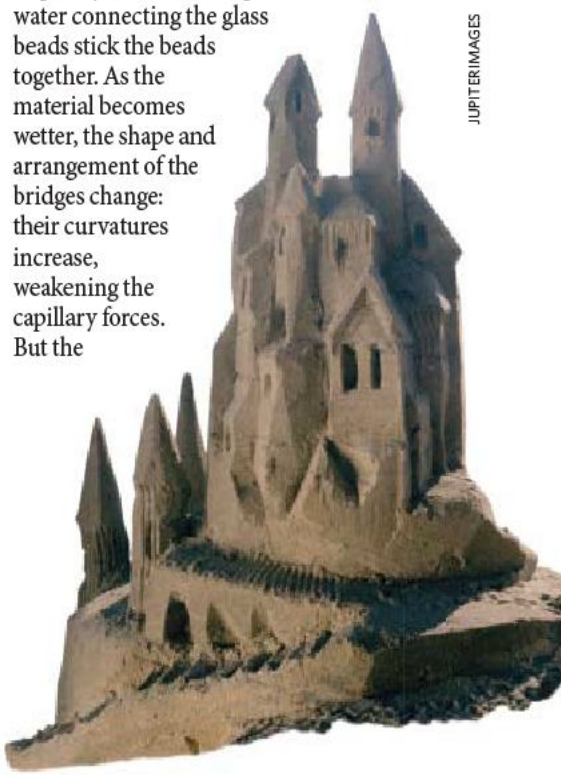
GRANULAR PHYSICS

Built on sand

Nature Mater. doi:10.1038/nmat2117 (2008)

The recipe for a sandcastle (pictured below) — just add water — is surprisingly insensitive to precise quantities. But why? Because two effects roughly balance each other in sand of almost any wetness, reseachers report.

Taking X-ray cross-sections of small glass beads of varying wetness allowed Stephan Herminghaus of the Max Planck Institute for Dynamics and Self-Organization in Göttingen, Germany, and his co-workers to monitor structural changes in 'sand' as they altered the water content of the mixture. Capillary forces in 'bridges' of water connecting the glass beads stick the beads together. As the material becomes wetter, the shape and arrangement of the bridges change: their curvatures increase, weakening the capillary forces. But the



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bridges reach more of the beads' surfaces, which helps the particles stay together. These opposing effects cancel out, so the sand's stiffness remains more or less constant whether a cupful or a bucket load of water goes into the castle.

ONCOLOGY

Melanoma mystery

Cell doi:10.1016/j.cell.2007.12.032 (2008)
Researchers have identified a protein that seems to underlie the paradoxical activity of a cancer-causing gene called *BRAF*. This gene can lead to melanoma but also causes pigment-producing skin cells to stop dividing and die.

The research group, led by Michael Green of the University of Massachusetts in Worcester, screened the entire human genome for other genes that could explain the mystery. They turned up 17, each of which could trigger *BRAF*'s killer activities. To their surprise, they found that a secreted protein, IGFBP7, is produced in cells with permanently activated *BRAF* genes, and acts to mute other signals induced by *BRAF* telling the cells to divide rapidly. Injecting IGFBP7 into mice with human melanomas slowed the growth of the tumours. The authors propose that loss of IGFBP7 allows *BRAF* to revert to its cancer-causing ways.

SPECTROSCOPY

Surface glance

J. Am. Chem Soc. doi:10.1021/ja710099c (2008)
By combining a spectrographic technique with a pulsed laser, researchers have developed a way to probe molecular interfaces such as biological membranes in real time.

To access very thin layers that are constantly moving, Jens Bredenbeck and his co-workers at the FOM Institute for Atomic and Molecular Physics in Amsterdam, the Netherlands, added a non-resonant laser pulse to two-dimensional infrared spectroscopy, which can be used to measure the properties of molecules in solution. The modification deletes the response from the bulk of a sample, leaving only information from molecules of interest — in their experiment, a thin layer of fatty alcohols in water.

Nuclear magnetic resonance spectroscopy has also been used to look at biological membranes, but it requires samples to be solid. The technique allows the molecular structure and behaviour of membranes in solution to be studied with femtosecond resolution.

IMMUNOLOGY

Primitive immunity

Nature Immunol. doi:10.1038/ni1562 (2008)
The kidneys and gills of lampreys — which, along with hagfish, are the only living jawless vertebrates — contain lots of cells that produce antibodies, researchers have found.

Max Cooper and his colleagues of the University of Alabama at Birmingham studied cells that carry antigen-binding molecules on their surfaces. The levels of these cells surged when anthrax spores were injected into the lampreys, but not when the creatures were injected with bovine serum albumin, to which more 'evolved' immune systems react.

After proliferating, the cells, which are thought to represent ancient B lymphocytes, differentiate and secrete a soluble, antibody-like protein, showing that lampreys are among the most primitive forms of life to express an adaptive immune response.

POPULATION ECOLOGY

Tabling more birds

Biol. Lett. doi:10.1098/rsbl.2007.0622 (2008)
Households that regularly sprinkle a few breadcrumbs and nuts on a bird table during winter are probably having a massive impact on bird breeding success in spring.

Gillian Robb and Stuart Bearhop at Queen's University Belfast in Northern Ireland and their team paired ten patches of deciduous woodland according to ecological and landscape features. They then simulated garden feeding (pictured below) in one of each pair by hanging wire mesh peanut feeders from trees from the start of November until early March. On average, birds at the supplemented sites laid their eggs earlier and raised nearly one extra surviving chick per nest than those in the unsupplemented sites, even though clutch sizes were the same.


PHOTOLIBRARY

JOURNAL CLUB

John Shepherd
Tyndall Centre for Climate Change Research, NOC, Southampton, UK

An oceanographer sees potential in accelerating rock weathering to soak up carbon dioxide from the air.

With CO_2 emissions increasing by more than 2% per year, rather than decreasing by the 3% or so needed to effectively mitigate climate change, I am not surprised that many scientists are seeking alternative solutions to simply cutting greenhouse-gas outputs.

Various geoengineering schemes have been proposed — such as fertilizing the oceans with iron, a limiting resource for planktonic algae that take CO_2 from the atmosphere — but these are unlikely to sequester large amounts of carbon in the long-term and may have serious ecological side effects. The thermodynamics of enhancing geochemical weathering look feasible, but the reactions are too slow to be really practicable.

Geochemists and engineers at Harvard University in Massachusetts and Pennsylvania State University recently suggested a kinetically preferable idea. They propose using the electrolysis of sea water to produce sodium hydroxide and hydrochloric acid, in a variant of the well-known industrial 'chloralkali' process, (K. Z. House *et al. Environ. Sci. Technol.* **41**, 8464–8470; 2007).

Sodium hydroxide could either be used to scrub CO_2 directly from the air, producing sodium bicarbonate, which is neutral and could be discharged into the sea, or be pumped directly into the ocean, increasing sea water's alkalinity and so its ability to absorb CO_2. The hydrochloric acid could be neutralized fairly easily, because it reacts rapidly with both carbonate and silicate rocks.

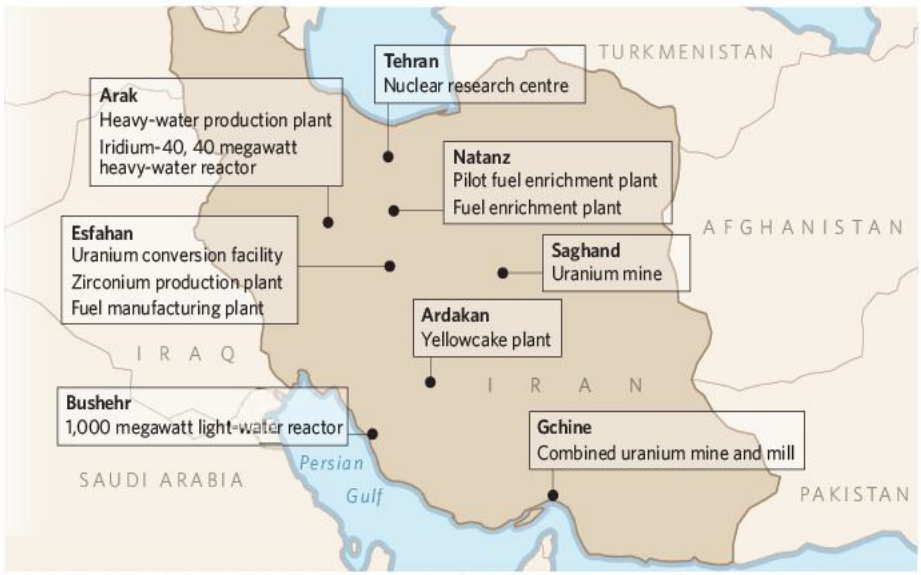
The scheme House *et al.* outline looks promising if it were operated using a solar or geothermal electricity source near a supply of basic rocks. A mid-ocean volcanic island would be good. And the environmental consequences of the scheme's discharges should be less severe than those of the ocean acidification that humans are already causing.

Discuss this paper at **http://blogs.nature.com/nature/journalclub**

NEWS

Atomic agency to give verdict on Iran

The International Atomic Energy Agency is wrapping up its inquiry into Iran's nuclear activities and is expected to report its findings on 20 February. **Declan Butler** analyses how close the state is to nuclear weapons capability.



DIGITALGLOBE

Natanz's enrichment complex with two large centrifuge halls visible in 2002 (left) but buried by 2004.

In 2003, Iranian opposition groups identified the physics research centre at Lavizan-Shian in northeastern Tehran as a clandestine nuclear facility. But by the time inspectors from the International Atomic Energy Agency (IAEA) got to the site in June 2004, Iran had bulldozed the facility to the ground, meticulously cleaned the site and removed all the topsoil.

Equipment from the centre later turned up at a university and IAEA inspectors found that it was contaminated with particles of highly enriched uranium (HEU). What work went on at Lavizan-Shian is one of numerous questions (see 'A mine of activity') to be addressed in a report by the IAEA due to be released later this month, and one that Iran has promised to answer after years of foot-dragging.

The IAEA has unravelled a long list of Iran's flagrant violations of IAEA nuclear-safeguard obligations. Iran failed to declare imports of 1,800 kilograms of uranium products from China in 1991. It neglected to tell the IAEA about uranium-enrichment activities using both gas-centrifuge and laser-enrichment techniques. And it failed to report research such as separating plutonium from irradiated uranium dioxide (UO_2).

From 1985 until 2002, Iran ran a covert nuclear programme based mainly on enrichment technologies it obtained from the black-market weapons network operated by notorious Pakistani scientist Abdul Qadeer Khan. Getting a clearer picture of what went on in the programme will help to clarify the nation's current nuclear motives, although the safeguard violations alone throw serious doubts on the alleged peaceful intentions of its nuclear efforts.

Last December, the US intelligence agencies issued a consensus estimate on Iran that concluded that Iran had stopped its weapons programme in late 2003. The estimate has been widely interpreted as a U-turn by the United States, but a key point in the text went almost unnoticed — that Iran had a weapons programme at all. This assertion has not been contested by either Iran, which has long denied

Break-out scenarios

Experts have come up with several scenarios under which Iran might develop a nuclear bomb. In one scenario, Iran continues to produce only low-enriched uranium, thereby meeting International Atomic Energy Agency (IAEA) safeguards, until it has sufficient stock to produce enough highly enriched uranium (HEU) for at least one bomb. It then withdraws from the nuclear non-proliferation treaty (NPT), expels IAEA inspectors and switches its enrichment capacity over to produce HEU — an operation that would take just weeks and would generate enough HEU for a bomb in 3–6 months.

Iran admitted last week that it has been testing a more advanced design of centrifuge, the IR-2, that will allow it to enrich uranium 2.5 times faster than it can with its existing P-1 centrifuge.

But given the threat of military strikes on Natanz, Iran would probably use the centrifuge expertise it has acquired to build a secret enrichment plant. For the past two years, the nation has denied the IAEA permission to inspect facilities other than its declared nuclear sites. In its last report on Iran in November 2007, the IAEA concluded that it was "not in a position to provide credible assurances about the absence of undeclared nuclear material and activities in Iran". That is because Iran is one of almost half of NPT member states that have not ratified extra powers given to the agency in 1997, such as short-notice inspections of any site it wants. Iran had been voluntarily allowing such broader access since May 2004 but stopped doing so in January 2006. The big question is whether a new report expected from the IAEA will reveal any substantive change here. **D.B.**

M. NIKOUBAZL/REUTERS

Shortly after Iranian president Mahmoud Ahmadinejad (left) met International Atomic Energy Agency chief Mohamed Elbaradei in January, he declared that Iran would continue its nuclear programme.

it had a weapons programme, or by the IAEA, which never explicitly stated as such — indeed, both welcomed the new US position.

Moreover, the estimate defined the "nuclear-weapons programme" as meaning covert nuclear enrichment, or efforts to build a nuclear warhead. The latter is ostensibly an easier step in building a nuclear weapon, experts say. The main obstacle to producing nuclear weapons is obtaining HEU or plutonium.

The US estimate did not cover Iran's supposedly civilian fuel-cycle facilities. Iran has ignored demands from the United Nations Security Council that it suspend enrichment and has relentlessly expanded its capacity at a plant in Natanz. It also continues to operate a heavy-water production plant and is completing a heavy-water reactor at Arak that can produce weapons-grade plutonium (see map).

Iran's current progress is cause for concern. Physicist Richard Garwin, who is a senior US government adviser on nuclear weapons and other security issues, last month calculated the low-enriched uranium (LEU) output of Natanz. He concluded that even if the plant operated at its planned maximum capacity of 54,000 centrifuges, this would be insufficient to fuel a nuclear power plant. But he noted that the 3,000 centrifuges currently installed at Natanz would be more than adequate for generating enough HEU for nuclear weapons.

Using just the existing centrifuges operating at levels stated by Iran, Garwin calculates that if the plant switched them to HEU and fed them with the LEU now being legally produced, it would be possible to make enough material for 61.6 kilograms of HEU a year — the equivalent of three bombs. The concern is that once Iran reaches these levels it might 'break out' and go for the bomb (see 'Break-out scenarios'). According to Andreas Persbo, a researcher at VERTIC, a nuclear verification organization in London, "this strategy was pursued successfully by Pakistan in the early- to mid-1990s, during a time when it had promised the United States that it would stop producing HEU".

If Iran were to break out, when would it happen? December's US intelligence estimate stated "moderate confidence" that the earliest possible date would be late 2009, but that this was "very unlikely" and it was more likely to be between 2010 and 2015. It could even be later than that because of technical problems such as impurities in the uranium feed and difficulties in operating banks of centrifuges. *Nature* has seen preliminary results from the European Commission Joint Research Centre's Theoretical Centrifuge and Cascade Simulator, a sophisticated model of the output of the current Natanz 3,000 centrifuge cascade under various configurations. One of the scenarios generated posits that if the cascade operated at full capacity, Iran could break out as early as July this year, with enough HEU for a bomb by the end of the year. Operating at only one-quarter of capacity, it would reach that point by 2010. ■

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www.nature.com/news

A mine of activity

The International Atomic Energy Agency (IAEA) has already uncovered violations by Iran of the IAEA's safeguards obligations and is due to report by the end of the month on unresolved issues, including:

The Gchine mine
Iran hid the Gchine uranium mine from the IAEA until 2004, and the IAEA wants an explanation of suspected military involvement in its running. Iran says that domestic uranium is key to achieving nuclear independence, but its annual output of 71 tonnes of natural uranium from both its declared mines falls far short of the amount needed to fuel a nuclear power plant. It is, however, more than adequate for a weapons programme. Iran also has a reliable supply of nuclear fuel from Russia.

The 'Green Salt' project
Information obtained by the US Central Intelligence Agency from a laptop computer in Iran discussed a 'Green Salt' project, named after the common name of uranium tetrafluoride (UF_4), a key intermediary in the production of uranium hexafluoride (UF_6) for enrichment. The documents described a possible clandestine processing plant and military connections, including tests related to nuclear weapons and missiles.

Polonium-210
The IAEA has detected undeclared work in Iran on polonium-210, an element with few applications other than as a trigger for nuclear weapons. Iran says that the polonium was being used for research on satellite batteries.

Uranium warhead hemispheres
IAEA inspectors found a 15-page document in Iran detailing how to reduce UF_6 to uranium metal, and to cast it into the hemispheres used in nuclear warheads. Iran has told the agency that it received the document by accident among documentation on enrichment centrifuges it received from the Khan black-market network in 1987. The IAEA is seeking clarifications from Pakistan. The agency has no evidence that Iran has produced hemispheres, but notes that it has declared "a small UF_6 to uranium metal conversion line in the Uranium Conversion Facility".

Given the scale of Khan's 'nuclear Wal-Mart', and the volume of material and documents it has supplied Iran with, it is possible that the document was like "one of those plastic submarines you get in a packet of cereal", says a source close to the IAEA inquiry. **D.B.**

Chinese astronomers look to Antarctic

A Chinese expedition returned last week from a 14-day crawl across the East Antarctic ice sheet in cargo containers, pulled by tractors, that doubled as living quarters. The trip, sponsored by the Polar Research Institute of China, completes only the second traverse to Dome A — the highest point on the eastern ice cap and the place where China intends to start building a research base next year.

The team also set up a suite of research instruments to study the atmosphere and sky above Dome A, most notably a remotely operated observatory called PLATO, which will assess how good the skies are for astronomical 'seeing'. PLATO includes four 14.5-centimetre telescopes, built in China, that will take advantage of more than three straight months of darkness during the Antarctic winter. "We think Dome A is the best site on Earth for astronomy," says Xiangqun Cui of the Nanjing Institute of Astronomical Optics and Technology.

The hope is that the desolate plateau, which sits 4,100 metres above sea level, will boast conditions unrivalled elsewhere on the planet — even at the French/Italian base at Antarctica's Dome C, 1,200 kilometres away, which set up its own automated test observatory in 2003 and has since ramped up to larger projects.

Proponents of Antarctic astronomy have looked to Domes A and C as alternative sites to the South Pole, above which 300 metres of turbulent air cause observations of stars to jitter and blur[1]. Dome C, by contrast, has only 30 metres of turbulent air above it, and less atmospheric interference than astronomical observing sites in Hawaii and Chile[2].

Although it lacks infrastructure, Dome A, which sits some 900 metres higher than Dome C, could be an even more promising site. "Each additional 100 metres is very important because it knocks out a huge chunk of the atmosphere," says Michael Ashley of the University of New South Wales in Sydney, which built PLATO. Models suggest that Dome A may have a turbulent layer as thin as 5 metres, he says, and thus even better seeing.

Cold comfort: China has set up a remotely operated observatory on Dome A, the summit of East Antarctica's ice cap.

Such conditions make Dome A attractive to Chinese astronomers, who have begun work on a suite of three 0.5-metre telescopes that they hope to deploy at the site in 2009. They are also eyeing the location for a potential US$40-million, 4-metre infrared and optical telescope. A proposal on that may be submitted this summer to the Chinese Academy of Sciences.

The true potential of Dome A may lie in observations outside optical wavelengths. The efficiency of infrared astronomy is particularly sensitive to temperature, and winter nights that drop as low as –90 °C will eliminate much of the noise from the atmosphere and the telescope itself, researchers say.

The potentially unparalleled dryness of the air may also allow astronomers to access parts of the electromagnetic spectrum that are obscured by water vapour elsewhere, including Dome C. "We're hoping to observe at very high radio frequencies that you would only otherwise be able to do in a plane or in space," says Craig Kulesa of the University of Arizona, who has an instrument on PLATO that will assess the transparency of the atmosphere. If conditions are as dry as expected, Kulesa hopes to deploy a telescope to Dome A that will map the Milky Way in the far infrared, to learn more about star formation.

But future projects will depend on PLATO, which researchers hope will yield meaningful data before next year's trip to refuel the station. "We already know Dome A will be the best spot on Earth," say Kulesa. "But the question is, how good is that?" ■

Rachel Courtland

Stars on ice

Most astronomical projects in Antarctica are based at the South Pole. They include:

The US 10-metre South Pole Telescope, which saw first light in February 2007, scans the sky in the millimetre and sub-millimetre range, focusing primarily on the cosmic microwave background left over from the Big Bang.

IceCube, the underground US neutrino observatory, reached its halfway point in construction this Antarctic summer; completion is set for 2011.

At the McMurdo Station in the Ross Sea, the United States launches payloads aboard scientific balloons reaching up to 42 kilometres. This year's missions include US and Japanese instruments examining cosmic rays and antimatter.

At the more recently established Dome C, projects include:

Italy's Small IRAIT, a 25-centimetre prototype for an 80-centimetre microwave telescope, was installed last year to study objects such as brown dwarfs in the Milky Way.

France's ASTEP, a 40-centimetre telescope dedicated to photometry and exoplanet searches, is scheduled to be mounted on Dome C in January 2009. **R.C.**

1. Travouillon, T. *et al. Astron. Astrophys.* **409,** 1169–1173 (2003).
2. Lawrence, J. S. *et al. Nature* **431,** 278–281 (2004).

Natural gas back in favour with US power companies

Electric utilities in the United States are quietly shifting their sights from coal to natural gas as the lower-risk fuel while they wait for the nation's carbon policy to be decided.

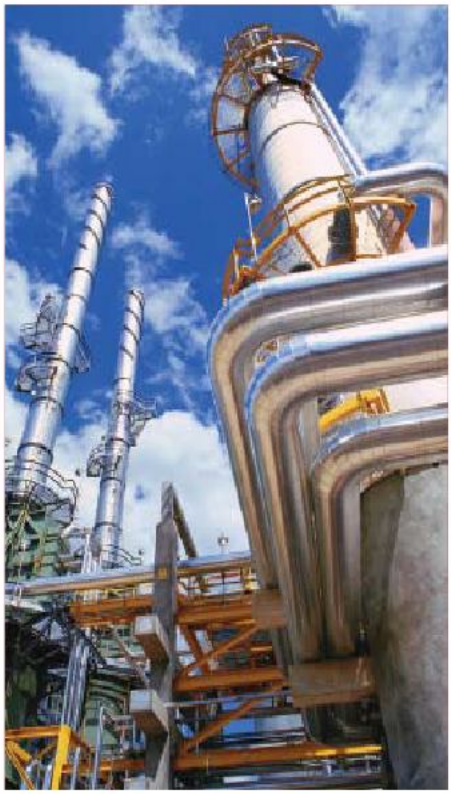
Recent proposals to build coal-fired power plants have been sunk after hitting a wall of public and political opposition in Texas, Kansas and Florida. Investors and lenders — including some of Wall Street's leading investment banks — are increasingly worried about the imposition of greenhouse-gas regulations in the coming years. Add to this the rising prices of materials such as steel and concrete, which are driving up construction costs, and coal no longer looks like the safe investment it did just a few years ago.

This new calculus hit home in 2007, when plans for more than 50 coal-fired power plants were cancelled, according to consulting firm Global Energy Decisions, based in Boulder, Colorado. In their place, experts say, utilities are turning to natural gas as a quicker, cheaper and lower-emission alternative to meet growing demand while lawmakers sort out the nation's carbon policy.

"Over the past year to two years there's been this 180-degree turn from concerns about dependence on natural gas to concerns about climate change," says Larry Makovich, an energy analyst and senior power adviser at Cambridge Energy Research Associates in Massachusetts. "The US power sector has started to move back towards natural gas in a very substantial way."

Cleaner-burning natural gas became the fuel of choice for new electricity generation in the 1990s, but increased demand coupled with peaking production in many US gas fields caused prices to spike in 2002. At about US$8 per million British thermal units, today's prices are more than triple the average throughout the 1990s. Building more natural gas plants could drive prices even higher — and make the nation that much more dependent on imports, which are already on the rise. Coal currently provides almost half of the nation's electricity, and is one of the few domestic energy resources that remains in abundance. Utilities also see coal, along with nuclear power and natural gas, as a critical source of constant 'base-load' power.

But given the major up-front costs and a lack of certainty about long-term economics owing to impending climate regulation, neither coal nor nuclear power look terribly attractive from an economic standpoint at the moment, says Revis James, a researcher at the Electric Power Research Institute in Washington DC. "In the long run, coal and nuclear will have to play a pretty big role," he says. "But for now, gas seems to be the default."



K. BURKE/CORBIS

Natural gas produces fewer emissions than coal, but supplies are shrinking and its cost is rising.

> "There's been this 180-degree turn from concerns about dependence on natural gas to concerns about climate change."

Dan Riedinger, a spokesman for the Edison Electric Institute, the industry's main trade association, acknowledges the trend towards natural gas but suggests that the expansion of coal-fired power is unlikely to halt. The resurgence of coal several years ago was "greatly exaggerated", he says, "and I think there is certainly some hyperbole with regard to the pendulum swinging in the other direction". ■

Jeff Tollefson

Keio Medical Science Prize 2008
Call for nominations

The Keio Medical Science Prize is awarded to researchers in recognition of their outstanding achievements in the fields of medical or life sciences. For 2008, the Keio Medical Science Prize Selection Committee welcomes medical sciences or life sciences related to the field of medicine.

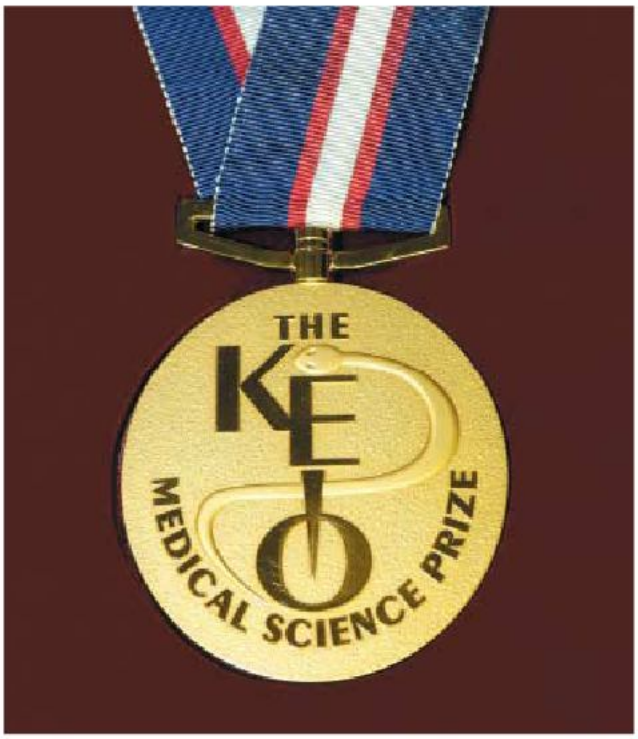
The award ceremony will be held in November, 2008 and the prize consists of a certificate of merit, a medal and a monetary award of 20 million yen (approx.US$180,000)

Application Deadline : March 20, 2008

To propose a candidate, please download nomination forms from our website and send it to Keio University Medical Science Fund.
(http://www.ms-fund.keio.ac.jp/prize/)

Previous laureates

1996	Stanley Prusiner, Shigetada Nakanishi
1997	Robert A. Weinberg, Tadatsugu Taniguchi
1998	Judah Folkman, Katsuhiko Mikoshiba
1999	Elizabeth H. Blackburn, Shinya Yoshikawa
2000	Arnold J. Levine, Yusuke Nakamura
2001	Tony Hunter, Masatoshi Takeichi
2002	Barry J. Marshall, Koichi Tanaka
2003	Ronald M. Evans, Yasushi Miyashita
2004	Roger Y. Tsien
2005	Yoshinori Fujiyoshi
2006	Thomas A. Steitz
2007	Brian J. Druker, Hiroaki Mitsuya



About Keio University Medical Science Fund

In 1994, Dr. Mitsunada Sakaguchi, a 1940 alumnus of Keio's School of Medicine, donated ¥5 billion to the university, expressly to encourage medical research and creative progress at the university and to promote worldwide medical advances. It was specifically intended that the donation be used to expand the network of medical researchers in the global academic community and to support them through grants.

To fully reflect Dr. Sakaguchi's commitment to medical progress, the university launched the Keio University Medical Science Fund on April 1, 1995. Dr. Sakaguchi made an additional donation of ¥2 billion, bringing the fund's endowment to ¥7 billion.

The Keio Medical Science Prize was established as one of the Keio University Medical Science Fund's major projects in order to contribute to the advancement of life science and medicine, and to encourage the expansion of researcher networks. It was the hope that by rewarding outstanding achievements in the fields of medical and life sciences, the prize will ultimately contribute to world peace and prosperity of humankind.

It is the only prize of its kind to be awarded by a Japanese university.

Award Ceremony of the Keio Medical Science Prize 2007 was held at School of Medicine, Keio University.

About Keio University

Keio was established in Tsukiji, Edo (now Tokyo) by Yukichi Fukuzawa, who is respected as one of the founding fathers of modern Japan. It dates back to the formation of a school for Dutch studies in 1858. Since the school's inception, the students of Keio have risen to the forefront of innovation in every imaginable academic field, emerging as social and economic leaders. Keio's School of Medicine was established in 1917 as the institute's fourth department.

Keio will cerebrate it's 150th anniversary in 2008.
http://www.keio.ac.jp/index-en.html

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Q&A

Lessons from the dark side

What role did German scientists and their funding organization play in supporting Nazi policies during 1933–45? In a bid to find out, Germany's research council, the DFG, sponsored a seven-year investigation into its own history that ended last month. One of the historians who led the study, **Ulrich Herbert** of the University of Freiburg, discusses the findings.

What sources were made available to you?
We were given access to all the DFG files, right back to the start of the society in 1920. These files were fundamental because we wanted to analyse what the scientists themselves requested funding for. Then we were able to look at what they did with the money they got.

What did you discover during the investigation?
We didn't find out about any sensational new crimes — and we weren't expecting to. Most of the immense dark crimes we know about today, such as the euthanasia programmes and human experimentation, were discussed after the Nuremberg trials that immediately followed the war. But we learnt how exquisitely closely 'normal' professors — not just the mad Nazi types — aligned their goals with the policies of the Nazi regime. Grant applications showed, for example, how many professors took part in developing plans for expansion to the east after Germany won the war (as they assumed). Plans that would have killed or enslaved more than 30 million people.

Did the effects persist beyond 1945?
Because we covered the period from 1920 to 1970, not just the specific Nazi years, we were able to document the impact on the German intelligentsia of the nationalism that arose after the First World War. We saw that it didn't end in 1945, but continued into the early 1960s, when there was a change of generation. We also saw that the expulsion of Jews and democrats from the universities — nearly a quarter of all professors — stymied the development of modern biology. After the war, molecular and cell biology took off in other countries, but Germany was left behind. On the other hand some subjects, such as cancer research, were unhindered. In fact, some very good science was funded by the Nazis — leaving Germany in good shape.

H. FRATER/DFG

Ulrich Herbert (inset) has examined the DFG's murky past.

What did you learn that may have relevance today?
The prevailing political view in Germany is that federalism is a better defence against totalitarianism than centralized power. The 1949 German constitution introduced extensive federalism for this very reason. But we saw that the behaviour of scientists at universities, which were decentralized in Nazi times, was no different from scientists working at the institutes of the centralized Max Planck Society, [which historians investigated a few years ago]. So we can't say that federalism alone is the answer to all evils.

Universities ended up colluding with the regime because the conservative professors who were able to continue working there agreed, like the general population, with most Nazi policies. There was no organized opposition to the views and no public debate of different positions. So although euthanasia of the mentally deficient was debated in many countries in the 1920s and 1930s, it was not only put into practice but accelerated in Germany. Having learnt that eliminating pluralism leads to radicalization, it's no wonder that Germany is cautious about things such as stem-cell research. We've experienced how things can go wrong in this sphere. Maybe we can be proud of the constant public debates we have, even though they seem to slow things down.

Do you think that other countries — Russia or China, for example — should undertake similar investigations?
I'm a bit shy, as a German, to say that others should learn from what Germany has done. But I think all countries with a totalitarian past might similarly gain from considering the history of their institutions — particularly their academic systems, where so much collaboration occurs. It's good to know the dark side of one's history — self-confidence is deepened if we are more aware of our own mistakes. It is also important to know if there are continuities — we found that many colluders made good scientific careers in our new democracy. Of course each nation has to decide for itself, but I think the exercise is very fruitful. ■

Interview by Alison Abbott

ON THE RECORD

"When a 4,700-pound pickup truck meets a 5,000-pound seal, they both lose."

Ken Cumings of the Friends of the Elephant Seal group in California describes the carnage when the huge beasts try to cross roads.

VALENTINE'S SPECIAL

A rose by any other hue
Blue roses are in production and will go on sale next year. Developed by Japanese researchers, the genetically modified blooms are likely to be pricey, so start saving.

Kiss and tell
Not just an expression of affection: kissing is also used subconsciously by both sexes to assess the status of their relationship, say researchers, who say it might also be addictive.

ROBOT NEWS

Robopump
Dutch inventors have developed a robot that can pump petrol, removing the tiresome need for drivers to exit their cars at a petrol station. The €75,000 (US$109,000) automaton finds a match for the vehicle on a database, unscrews the fuel cap and fills the tank.

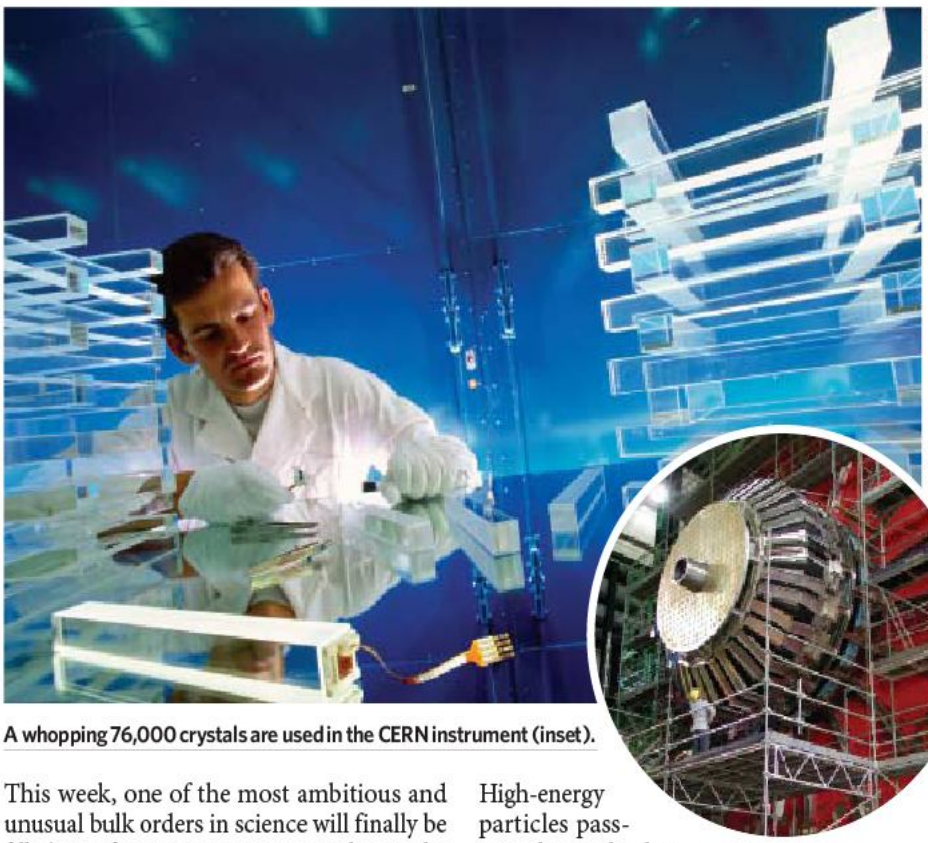
ZOO NEWS

Breath of life
Germany's love of cute baby animals — nicely portrayed by the ongoing Knut shenanigans — has reached a new level. Noticing that a tiger cub had choked on a piece of meat, medical student Janine Bauer climbed into its cage and resuscitated it at Halle Zoo. In gratitude, the zoo has named the tiger after Bauer's son Johann.

Sources: Los Angeles Times, AFP, Scientific American, Spiegel, Reuters

SIDELINES

From Russia with scintillation



P. GINTER/BILDERBERG

CERN

A whopping 76,000 crystals are used in the CERN instrument (inset).

This week, one of the most ambitious and unusual bulk orders in science will finally be filled. At a former Soviet weapons plant in the Russian town of Bogoroditsk, workers will pull from one of their 159 ovens the last of thousands of highly specialized crystals being produced for the Compact Muon Solenoid (CMS). The CMS, a scientific instrument the size of a building, is being assembled at CERN, the European particle-physics laboratory outside Geneva in Switzerland.

"This indeed is the end of a chapter," says Tejinder Virdee, who leads the CMS group at CERN. It is a chapter that began 14 years ago and is ending in the nick of time — the electromagnetic calorimeter for which the crystals have been made must be completed before CERN's Large Hadron Collider (LHC) is switched on in June.

"When we started, energy in Russia was essentially free and salaries were low."

The CMS is one of three detectors that will study the particles given out when the extraordinarily powerful beams of protons provided to the experiment by the LHC smash into each other. The electromagnetic calorimeter's role is to measure the energies of particles coming off the collisions at the detector's centre. High-energy particles passing through the calorimeter create showers of secondary particles as they interact with the crystals, and these showers in turn produce light in proportion to the energy of the original particle. "The whole point of the crystal is to convert energy into light," says Philippe Bloch, a physicist at CERN working on the project.

There are various different sorts of crystal that might serve, but in 1994 the CMS team decided that scintillating lead tungstate ($PbWO_4$) would best serve their needs. As transparent as glass but nearly four times as dense, it allows a compact, radiation-resistant detector that responds quickly to incoming particles. And the showers of secondary particles within it do not spread out too much, making it easier to discriminate between different events.

The problem was that the more energetic the particles produced in a collider, the bigger the detectors need to be. When it is turned on, the LHC will work at higher energies than any previous machine, and the CMS and its fellow

SKYSAILS

SHIP KITES INTO PORT
After the maiden vogage of the SkySails cargo ship, *Nature News* examines towing ships with kites.
www.nature.com/news

detectors need to be correspondingly colossal. The electromagnetic calorimeter — one of the smaller components — is 7 metres long and 4 metres across, weighing in at around 100 tonnes. It requires 76,000 crystals — more lead tungstate than all the previous particle detectors in the world combined.

Making the crystals is no easy feat. For starters, each one must sit in a platinum-lined crucible heated to 1,440 kelvin for two-and-a-half days. No factory in Europe was capable of making so many crystals, even with more than a decade's lead-time. So Paul Lecoq, who was in charge of the search for a production site, looked east. The Russians had for years grown crystals en-masse for their radars and missile guidance systems. "They have grown everything you can dream of," Lecoq says. And the former Soviet Union built production facilities on a scale far beyond those anywhere else. In December 1994, Lecoq found himself inside a once secret underground plant for growing crystals in Bogoroditsk, around 300 kilometres south of Moscow. The sheer size of the facility stunned him: "It was something I could not have imagined."

"This was a massive logistical activity. It's not the best way to do it, but it's the only way we could."

Using money set aside for weapons non-proliferation by Western powers, LeCoq and colleagues set about fixing broken ovens and rehiring staff for the factory. The Russian government loaned the collaboration platinum from its reserves, and over the next four years, workers learned how to grow the crystals.

But there were problems. The Russians struggled to obtain the high purities that the CMS requires to obtain accurate data. And Russia itself was changing. "When we started, energy was essentially free and salaries were low," says Bloch. Towards the end of the 1990s, the cost of labour and energy rose dramatically, and the price tag for the crystals tripled, bringing the cost of the calorimeter to US$110 million.

Efforts to streamline production floundered, and to make up for lost time, the team was forced to employ a second factory in China. Even then they struggled to stay on schedule. Indeed, if the start-up date for the LHC itself had not been delayed — it was meant to circulate its first beams in 2007 — it is unlikely that the CMS calorimeter would have been ready in time. As it is, even with the last crystal due out this week, the calorimeter will not quite be finished by its end of May deadline, although it should be good to go soon after. "We are a little bit late, it's true," says Bloch.

Such delays, though, are almost to be expected in a $480 million project that seeks to combine bespoke parts from all over the world. The vast steel support structure for the CMS comes from a shipyard in Hudong, China; the pipes in its cooling systems are Polish; its radiation-hardened electronics were built in the United States, France and the United Kingdom. All told, 38 countries have contributed parts to the detector.

"This was a massive logistical activity," says Virdee. "It's not the best way to do it — but it's the only way we could." ■

Geoff Brumfiel

Biofuels might create more emissions than they save

The use of biofuels could sharply increase greenhouse-gas emissions by driving the conversion of native land to agriculture, according to research published online last week in *Science*.

Timothy Searchinger from Princeton University in New Jersey and his colleagues suggest that it takes 167 years for the emissions reductions gained by burning corn ethanol to make up for the sharp spike in emissions caused by clearing new land for farms (T. Searchinger *et al. Science* doi:10.1126/science.115861; 2008).

And a second study, led by Joseph Fargione of the Nature Conservancy in Minneapolis, Minnesota, calculates that the time needed to clear this 'carbon debt' would vary from 17 to 420 years, depending on the circumstances (J. Fargione *et al. Science* doi:10.1126/science.1152747; 2008).

But biofuels made from waste biomass, or perennial crops grown on abandoned agricultural lands, offer "immediate and sustained greenhouse-gas advantages", according to Fargione's team.

Collectors claim trade treaty is obstructive

The 1973 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) restricts trade in endangered organisms, and the word among scientific collectors is that it restricts their research as well. A paper now illustrates this 'CITES effect' in hard numbers (D. L. Roberts and A. R. Solow *Proc. R. Soc. B* doi:10.1098/rspb.2007.1683; 2008).

David Roberts of the Royal Botanic Gardens in Kew, UK, and Andrew Solow of the Woods Hole Oceanographic Institution in Massachusetts, compared the rate at which two botanical gardens in the United States collected two kinds of plant: orchids, many of which are covered by CITES, and bromeliads, of which very few are. They found that before the convention was ratified, the institutions were collecting three orchids for every bromeliad, but after this time the ratio fell to just 1:1.

P. CRIBB

Botanical gardens are shying away from orchids.

Europe's lab finally arrives at the space station

NASA/ESA

The Columbus research module, Europe's largest contribution to the International Space Station, has finally arrived at its destination.

On 11 February, astronauts Rex Walheim and Stan Love conducted a spacewalk to connect the module to the station (clockwise from top left: Columbus is lifted from the shuttle, manoeuvred into position and docked onto the space station). Astronauts will now begin preparing the €700-million (US$1-billion) lab for experiments in biology, fluid science and physiology. A second spacewalk scheduled for 15 February will attach a materials-testing experiment and a solar observatory to the outside of the lab. But many larger experiments will be delayed until 2009, when the station's crew is set to increase to six (see *Nature* **450,** 766–767; 2008). The arrival marks the end of years of delays for Columbus, which was originally set to launch around 2000.

Illumina unveils genome sequence of African male

Illumina, a biotechnology company based in San Diego, California, announced on 6 February that it has sequenced the complete genome of an African man.

Scientists have already sequenced the genomes of two Caucasian men and one Chinese man, but Illumina's is the first African genome. It came from an anonymous Yoruban man from Nigeria.

Comparing the data from these four genomes might theoretically yield insights into human diversity. But data from only two of them have been publicly released — those of the scientists James Watson and Craig Venter — and only Venter's genome has been described in a formal publication.

The Illumina announcement came two days before company scientists unveiled their data at the Advances in Genome Biology and Technology conference in Marco Island, Florida.

Head of US environmental health agency resigns

The head of the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, has resigned. David Schwartz stepped aside temporarily last August when an investigation was opened into his management, in part because Congress had questioned him over potential favouritism and having conflicts of interest (see *Nature* 448, 979; 2007).

"Our community has not universally embraced the scientific direction or strategies that I have implemented," Schwartz wrote in an e-mail to staff. "In my enthusiasm to bring new science and opportunities to our field, it appears that I have inadvertently disenfranchised segments of our community. I sincerely apologize for the pain this may have caused."

Schwartz will now run the pulmonary and critical-care unit at the National Jewish Medical and Research Center in Denver, Colorado; he will also help to create a centre for genetics and therapeutics there.

Cash crisis puts Sunshine Project in the shade

After eight years, the Sunshine Project, an influential US biosecurity-monitoring group based in Austin, Texas, has suspended its operations.

The project's director, Edward Hammond, says he made the decision because of a chronic cash shortage. "At some point you come to realize that if you don't have buy-in from the people whose business it is to fund peace and security non-governmental organizations, then you have to recognize reality," he says.

Hammond built a reputation for being well-informed as he exposed numerous lapses in biosafety at US universities and research institutes while the country's investment in research on dangerous pathogens ballooned. He was a strong critic of what he alleged were lax controls on rules governing research on pathogens.

NATURE|Vol 451|14 February 2008

You have 4 minutes to choose your perfect mate

What is the secret to finding the right partner? Two researchers are using unconventional techniques to find out. **Matt Kaplan** investigates the science of speed dating.

Eli Finkel and Paul Eastwick have probably seen more first dates than most. The social scientists at Northwestern University in Evanston, Illinois, have watched hundreds of videos of single people as they participate in a curious, but not unpopular, trend known as speed dating. Two participants spill their souls to each other for a set time, say four minutes, and try to decide whether they might have a future together. When the time is up, they move on to a new partner, sometimes talking to a dozen or more people in a night.

Finkel and Eastwick, who often share play-by-play accounts of the videos they review, have seen enough exchanges to know when one dater, whom we'll call Dan, might blow it. Dan has just transferred to Northwestern and his date, 'Danielle,' asked if he was enjoying the social life at his new university (for those of you who don't speak fluent baseball, a translation of this exchange can be found at the foot of the page):

Dan (whispering and looking around to make sure nobody is watching): Honestly? I'm speed dating right now, *that's* how much fun it is.

Danielle (laughing): Come on, that's like a dis to the people that are here, regardless.

(*Finkel:* Oh that's such a hanging curveball.
Eastwick: Come on dude, swing!)*

Dan: No … you're awesome. I'm just saying … you know what I mean, though.

(*Finkel:* Nice! Handled the moment like a master.)

Danielle (smiling): No, OK, I understand. Yeah, definitely.

Dan may not be the slickest of operators, but by taking the chance to pay Danielle a compliment he is showing signals that could mean the start of successful relationship, say Finkel and Eastwick. "Showing unique liking to someone is an effective way to get them to like you," says Eastwick.

From a purely biological standpoint, the success of a partnership hinges mainly on one thing, reproduction. But for humans, who give birth to exceptionally weak, awkward and totally dependent babies, strong pair bonding and the sharing of parental duties can play an important part in the success of their offspring. It is strange, then, that a goal as simple as forming a pair bond could lead to an emotion as complex as romantic love.

Poets and philosophers have plumbed the depths of this feeling for generations, yet are still searching for answers. What do we see in a potential mate that makes our heads spin and our hearts flutter? What do we want in a long-term lover, and do our long-term wants govern our short-term passions? Do men and women want the same thing? Or for that matter, do they even know what they want?

Love will keep us alive

Since the 1940s, social scientists have brought the tools of their trade to bear on such lofty questions. Finkel and Eastwick are now using some of the newest and most controversial techniques. The fast-paced format of speed dating could be exhilarating, daunting or perhaps even dorky for participants and observers alike. Nevertheless, the researchers say that it could help to reveal some of the mysteries behind that uniquely human emotion — love. Indeed, their research, including a paper published today[1], has already started to turn up some surprises.

In the 1940s, when scientists first started to pick at the basis of human attraction, psychologists interviewed single people and asked them

*A hanging curveball is a slow-moving, easily hit ball — something that a batter should certainly swing his bat at …

what they would value in a partner. Many of the values were the same in both men and women, but two things stood out in survey after survey. Women valued the wealth of their partner much more than men did, and men valued attractiveness more than women did[2,3].

These differences can even make sense in evolutionary terms. A woman looking to have children would want the support of a good provider to help her children succeed in life. Men's seemingly superficial preference for beauty was seen as a proxy for health. Symmetry, skin tone and a favourable waist-to-hip ratio could reasonably point to a woman who would not only survive childbirth, but also pass on lots of healthy genes.

Yet, this is clearly not what drives the actions of everyone looking for love. Take Bob, a trombonist, and his speed-date Veronica.

Veronica: So, trombone …

Bob: I'm hoping that when I get out [of] here, I can do some work on a cruise line.

(*Finkel:* That almost certainly doesn't pay very much.)

Veronica (exploding with enthusiasm): That would be an amazing job!

(*Eastwick:* Yet she likes him anyway!)

Bob: Yeah, I think so too, I want to get out and do some travelling if I can. And then afterwards get into some studio recording work. 'Cause I'm addicted to movie soundtracks.

Veronica: Oh my gosh! I LOVE movie soundtracks!!!

(*Finkel:* Here we go with the unique liking again. This is a really good sign. *Eastwick:* So much for earning prospects.)

So how do researchers go about getting to the root of such behaviour? For many psychological questions, quality data can be collected by dragooning college undergraduates into an experiment for course credit. Even studies on relationships are relatively easy because many couples are eager to take part. The trouble with studies on initial romantic attraction is that they require the right people in the right place at the right time. Finkel and Eastwick thought that speed dating might given them a way around this logistical complication.

The pair was first inspired to use speed dating for research purposes in 2004, when Finkel was running a graduate course on how relationships develop. At the time, there was a reasonable understanding of what made long-term relationships satisfying or dissatisfying and a few studies had looked at initial attraction in live interactions. But "we didn't really know [what makes] people move from being perfect strangers to being lovers", says Finkel. Eastwick, then a student in Finkel's class, suggested that a speed-dating study might be a good way to study the transition because they could look at how well 'gut reactions' predict the course of a relationship. The two decided that an immersive field trial was in order, and went speed dating themselves.

"It was amazing how quickly we were making judgements."
— Paul Eastwick

Finkel and Eastwick hit the town and were immediately impressed by the dynamics of the fast-paced dates. "It was amazing how quickly we were making judgements about the people we were meeting," notes Eastwick. "We immediately recognized the scientific potential," adds Finkel.

So they crafted their own speed-date experiment. They recruited 163 undergraduates on campus to sign up for the study and complete a 30-minute online survey that explored their preferences in mate attractiveness, earning potential and personableness. At the event, participants were photographed and led through the speed dates as per usual, with one exception. In a short break between dates, the participants were asked to rate the person they just met on the preferences they had indicated in the survey. Then, when they went home, they filled out an online form saying whom, if anyone, they wished to be matched with, or in the language of the team's articles, who they would 'yes'. If two participants 'yessed' each other, they were informed and asked to rate, over the course of a month or more, their impressions of that person and any interactions they had with them[4]. The surveys allowed the team to monitor relationships as they bloomed or failed — tracking dates, feelings, anxieties and sexual encounters. Even if another love interest enters the picture during the course of a speed-date initiated courtship, the participants indicate them as 'write-ins', who are again rated by attractiveness, earning potential and how personable they are.

S. ALAIR

Eli Finkel (left) and Paul Eastwick are experts on dating.

Nothing compares to you

But not everyone is impressed with the idea. "Speed dating is a highly artificial situation," says James Giles, a philosopher at the University of Guam in Hagåtña and author of *The Nature of Sexual Desire*. Speed dating, he says, is unlikely to give insight into how normal romantic relationships develop. By depending on people who did not mind — and perhaps even enjoyed — being watched by strangers, they are analysing the behaviour of a very select group that may not have been representative of the wider population, explains Giles.

Although it is a contrived situation, Finkel and Eastwick defend the activity. Nearly everyone has experience of mixing with new people for the first time — at parties, clubs and the like. "A speed-dating event is like a party with a little bit of structure," says Eastwick, "a party where you are guaranteed a few minutes with the most desirable person in the room." "For that reason alone," Finkel adds, "speed dating might appeal to a broader subset of people than those who typically volunteer for psychology experiments."

Of course, the most pertinent feature of the speed date — its speed — might not

seem ideal for making such important decisions. If individuals don't have enough time to evaluate a potential mate, their impressions of mate qualities could be too unformed. Yet research has indicated otherwise.

Fifteen years ago, Nalini Ambady, now a psychologist at Tufts University in Medford, Massachusetts, ran a study in which she showed people silent videos of lecturers teaching and asked them to rate the quality of the lecturer. The clips she used were short, just two seconds long, yet viewers judged the abilities of the teacher with frightening accuracy — students who had taken classes by the lecturers for months posted the same assessments. "It was really surprising how good [the viewers] were at making accurate observations," says Ambady.

The idea that people can make accurate judgements about situations or individuals with a seemingly small amount of information — called 'thin-slicing' — is well supported in the literature.

In similar experimental demonstrations of thin-slicing[5], the more participants thought about their decision, the less accurate their judgements became, probably because the extra rumination complicated the decison-making process. The findings would seem to be a positive sign for the power of speed dating, suggesting that enough information can be gathered in a four-minute date to decide whether the person across the table might make a good mate.

Yet remarkably, Finkel and Eastwick have found that speed daters in their experiments often don't pursue people they would consider their ideal, or at least not the types of people they say they are most attracted to. Veronica from the trombone tale, for instance, was in the top 10% of women for how highly she rated her preference for good career prospects. And despite the fact that there were seven economics majors and two future doctors in the room that night, she was drawn to Bob, a trombonist, with low prospects for a lucrative career. They 'yessed' each other when they got home.

> "Speed dating is a highly artificial situation."
> — James Giles

More than a feeling

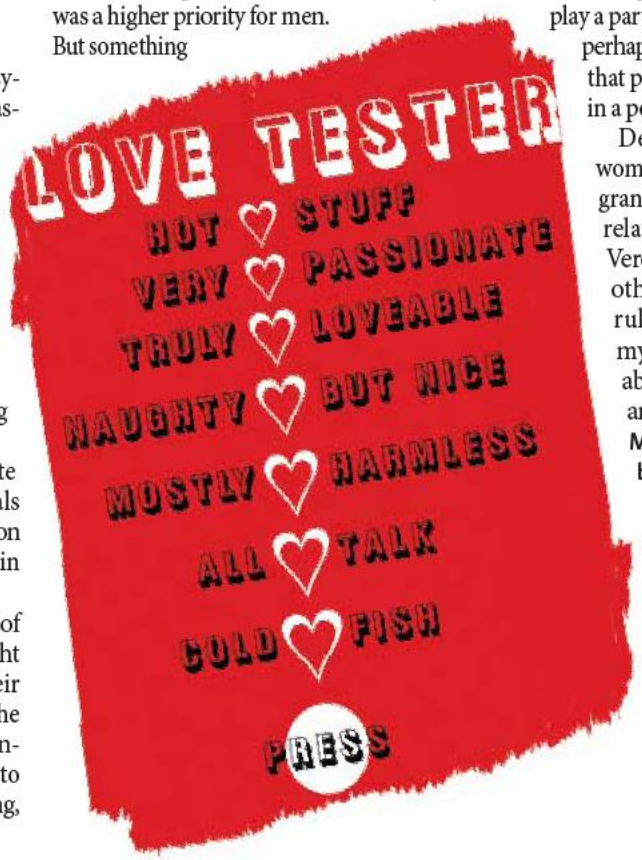
Finkel and Eastwick's latest research also suggests that the theory that women prefer money and men prefer beauty carries little weight, at least in initiating romantic relationships. In a study of 163 students from Northwestern University[1], Finkel and Eastwick set up a series of speed-dating events and before the event asked the students to describe what they were looking for in an ideal partner. Predictably, perhaps, these pre-event ideals mirrored what generations of papers before had predicted. Wealth was more important to women; beauty was a higher priority for men.

But something interesting happened when the students started reporting back about the relationships they were initiating. They hadn't acted on their stated preferences. In some cases, women even enjoyed their dates with attractive partners more than men did.

So ultimately, in addition to being a many splendoured thing, love is driven by the context. "Traits don't seem to jump out in isolation during real, social interaction," says Finkel. "By analogy, imagine I ask you whether you like eggs, and you say no. Does that mean you will prefer cake made without eggs? No, you evaluate the cake as a whole." Bob the trombonist is no different. The isolated idea of earning little money is poles apart from earning little money while pursuing a dream.

"The 'earning-little-money' ingredient when mixed with other ingredients, in this case passion and adventure, changed the final product. We think that's how people evaluate each other as romantic partners — as whole cakes not as individual ingredients," says Eastwick.

The results seem to contradict the evolutionary interpretation. Or at the very least, they suggest that we aren't particularly good at describing what we want. Nevertheless, just because the values people say that they are searching for don't match up with their desires in the moment doesn't make those values meaningless. An alternative explanation, Finkel and Eastwick suggest, is that the values simply do not play a part in the beginning of a relationship, perhaps being more important later on, or that people's assessments of those values in a potential mate are somehow flawed.



Despite the confusion, men and women will probably carry on with the grand experiment. Dan and Danielle's relationship is flourishing. Bob and Veronica, although interested in each other, haven't dated seriously. The rules of love and attraction remain mysterious and a little unpredictable. Of course, who would have it any other way? ■

Matt Kaplan is a science journalist based in London.

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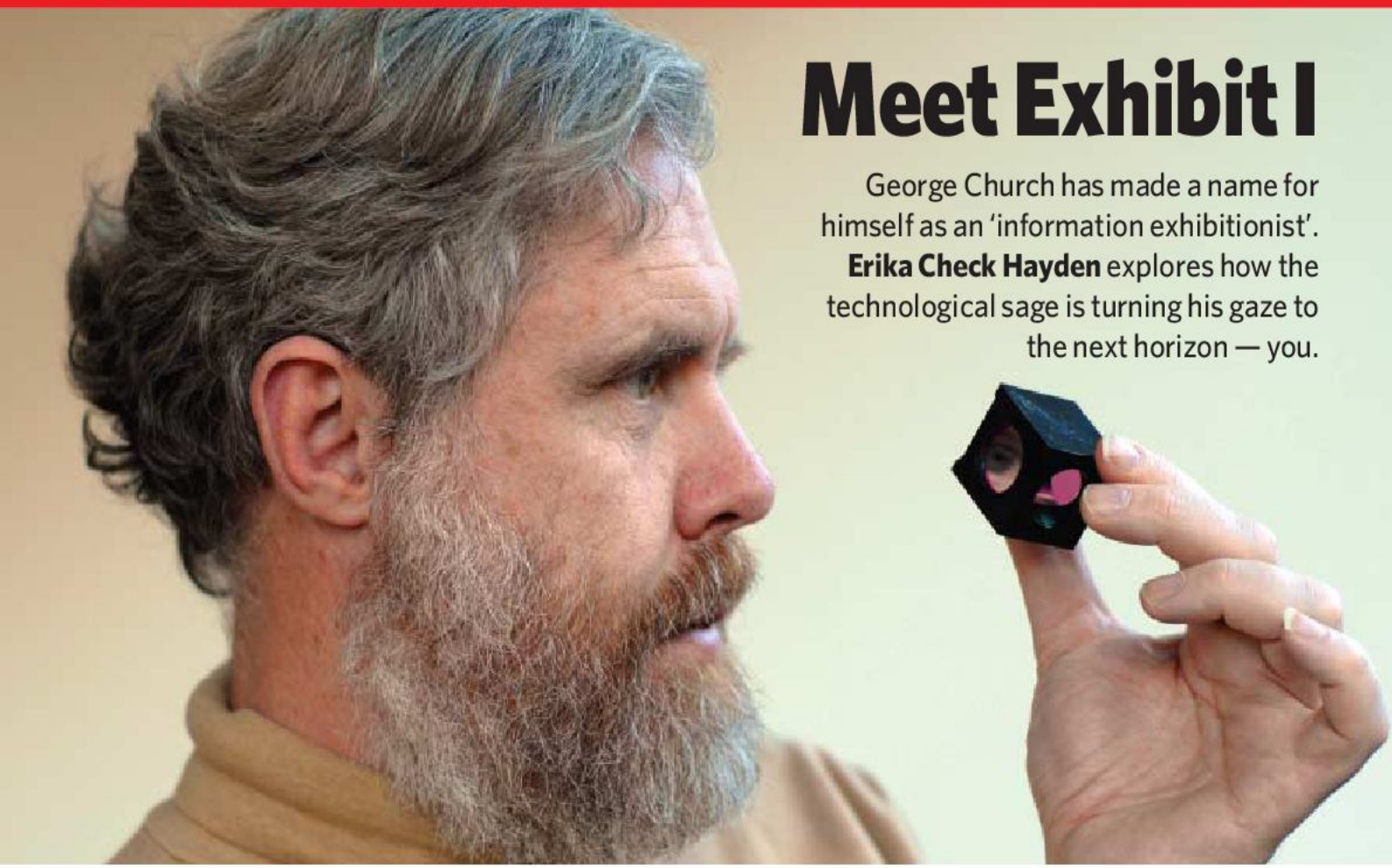
Meet Exhibit I

George Church has made a name for himself as an 'information exhibitionist'. **Erika Check Hayden** explores how the technological sage is turning his gaze to the next horizon — you.



S. OGDEN

One day in the 1980s, while a graduate student at Harvard University, George Church decided to change the way he saw the world. He started with simple tools — scissors, cardboard, and sticky tape — and he ended up working at his bench with a pair of homemade blinkers affixed to his temples. Asked about the nearly forgotten episode, an older, wiser, now-tenured Church chuckles at his younger self, but he still defends the stunt's essential point. "I was making a statement," he says, taking a break from running one of the largest, most eclectic biology labs in the United States. "Sometimes scientists put on blinders and don't see the big picture."

Performance art's loss was science's gain, but Church has never lost sight of the point he was trying to make that day — indeed, he has spent his career scanning the horizon for the new, the different and the maybe-just-possible. As biology has become ever more specialized, Church has continued to widen the scope of his work. Yet he brings to each new project a depth and intensity of vision that suggests he knows when to put the blinkers on as well as when to take them off.

Church spends most of his time dreaming up far-fetched ideas and doggedly pursuing them. His thoughts on DNA sequencing, first formulated decades ago, led to some of the high-output sequencing techniques now revolutionizing biology. His inquiries have led him to jointly found Codon Devices in Cambridge, Massachusetts, one of the first synthetic-biology firms. He has also founded two other companies, LS9 near San Francisco, California, which makes biofuels, and Knome in Cambridge, Massachusetts, which sells personal-genome sequencing services.

Some of Church's ideas don't work out. But many do. And it's his willingness to take risks that colleagues say is his biggest contribution to science. "He has a certain breadth to his knowledge," says Robert Weiss, a genomics scientist at the University of Utah in Salt Lake City, who has known Church since the 1980s. "He can cross disciplines successfully and integrate ideas to move a field forward, and he has done that repeatedly."

Open access

A tall and soft-spoken vegan whose salt-and-pepper beard lends him the aura of a mountain sage, Church's placid manner conceals a tendency to go to extremes to defend his convictions. Last year, when former Massachusetts Institute of Technology (MIT) biologist James Sherley felt that he was denied tenure due to racial discrimination, Church was among a handful of scientists who protested outside the MIT president's office in Cambridge. And Church has posted his birth date, mother's maiden name and signature on his web page to make the point that information wants to — and should be — free. His wife convinced him to remove his social security number, but other information, such as a map of the route he walks each morning to work, stayed up. Fritz Roth, a former student who now runs his own lab at Harvard Medical School in Boston, Massachusetts, calls him "the information exhibitionist".

> "Sometimes scientists put on blinders and don't see the big picture."
> — George Church

The title certainly seems apt for a current venture: the Personal Genome Project. Church has already begun sequencing portions of the genomes of ten volunteers: himself, Sherley, entrepreneur and futurist Esther Dyson and seven other individuals, six of whom have also revealed their identities. But unlike other large individual sequencing projects under way, such as the Chinese Yanhuang Project and the International 1,000 Genomes Project, Church will also collect information about his study subjects' 'phenotypes' — traits such as eye colour, height and medical conditions — and study how genes contribute to these phenotypes. Then he will publicly release the data in stages and study how this affects the participants, the practice of medicine, and society at large.

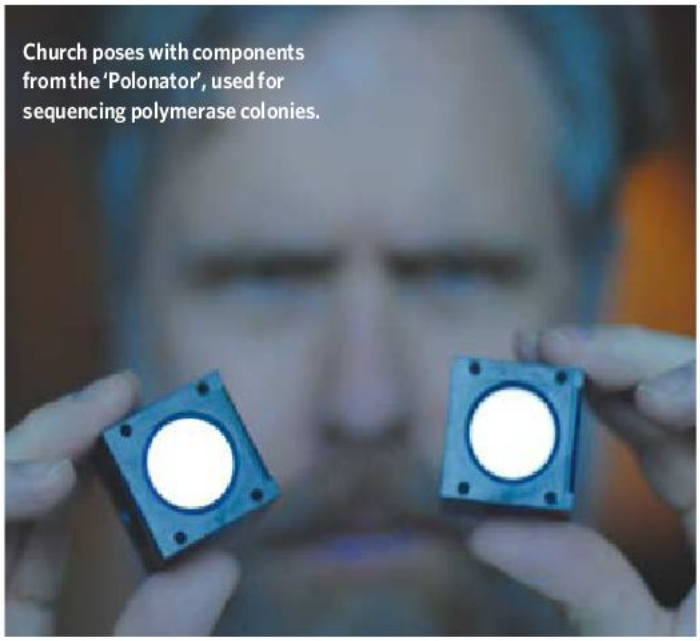
Along the way, Church plans to continue refining DNA sequencing and analysis in the hope of attracting more volunteers — perhaps thousands or millions. The project is a culmination of everything that preoccupies Church: technology development, large-scale biology, data transparency and the public interpretation of science. Society at large, he says, doesn't really know how to handle most genetic information. "I'm trying to make sure that there's enough information at a low enough risk so that all of the stakeholders in this technology, now or in the near future, will have a test set to work with," Church says.

Church's project has roots going back decades.

Church had had an aptitude for engineering even as a child. Yet he was inspired to apply that talent to biology by his second stepfather, a doctor Church refers to as his "third father". "I figured that everything in his black bag, such as antibiotics, and in his office, such as ultrasound, was invented by someone — maybe like me," he says. In his twenties, Church typed all the known nucleic acid sequences into a computer in one afternoon to make predictions about how corresponding RNA sequences might fold — an exercise that made him wish it were possible to know the DNA sequence of every person on Earth.

In 1976, he was kicked out of the biochemistry department of Duke University in Durham, North Carolina — he thought his time in graduate school was better spent publishing papers on RNA folding than on attending class — and found his feet at Harvard University in 1977. There, he sought out a like-minded mentor in Walter Gilbert, a biochemist who had developed one of the two standard methods for sequencing DNA. "There just weren't a lot of engineers in biology back then, and his lab seemed like an oasis where you could do technology and get away with it," Church says.

In 1980, Gilbert's sequencing work earned him the Nobel Prize in Chemistry, which he shared with Paul Berg and Frederick Sanger. Sanger had developed the other foundational DNA sequencing strategy, which was — and still is — the basis for most sequencing projects. Not long afterwards, Church and Gilbert joined a series of discussions on the possibility of a Human Genome Project, an audacious plan at the time, considering not even a simple microorganism had been sequenced.



S. OGDEN

Church poses with components from the 'Polonator', used for sequencing polymerase colonies.

You get what you pay for

Sequencing was a labour-intensive, error-prone process that cost US$10 per base — which meant that sequencing the whole human genome would cost $30 billion. The process needed to become a lot cheaper and faster, and the question was how — and if — that could happen. Church thought that the project should aim big. He advocated a strategy called 'multiplex' DNA analysis, one that he had developed and refined, partly with Gilbert's help. The strategy involved anchoring pooled DNA to a solid base, then exposing the DNA to a series of reagents to produce image readouts, all on the same machine — an approach that Church thought should save time, reagents, human effort, and money.

Setting up his first lab in 1986, Church worked to develop his concept. He was up against tight competition. Other labs were working on multiplexing, and the company PerkinElmer, now based in Waltham, Massachusetts, was investing industrial dollars on a sequencing machine developed by Applied Biosystems in Foster City, California. The machine improved and automated the traditional Sanger sequencing method.

In the mid-1990s, the US Department of Energy ran a comparison between the Applied Biosystems machines and other sequencing techniques, including Church's. Applied Biosystems won. Its sequencers were able to deliver finished genomes faster than multiplexing, and the company's machines became the workhorse of the Human Genome Project for the next decade.

Church was disappointed but he didn't give up, predicting that Applied Biosystems' sequencer would never be cheap enough for mass use. "You tend to get what you ask for, and if you ask for a genome for $3 billion it will be very hard to do a thousand genomes with the technology that can do that," Church says.

Plugging away throughout the 1990s, Church deployed some outlandish ideas. One project, for example, aimed to sequence DNA by passing it through a membrane protein channel while measuring changes in the flow of ions caused by differences in the size and charge of individual DNA bases. "Where many people would say this is practically not feasible, he would say, 'If you can't tell me what law of thermodynamics it violates, I don't see why we can't do it'," says Roth, who took on the project as a student. "I wouldn't say it failed utterly," he notes, pointing out that another Harvard lab is still working on the method.

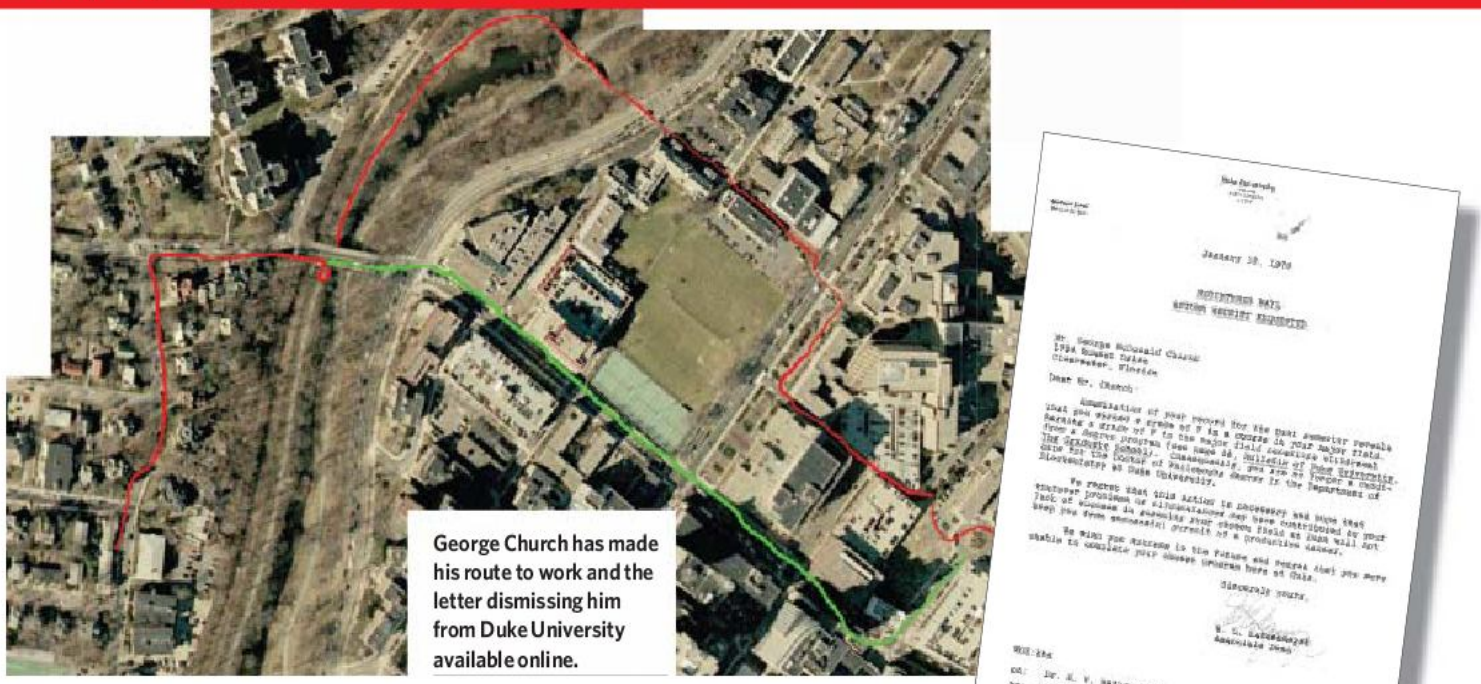
As the lab grew, Church gained a reputation for his cutting-edge ideas and eccentric personality. There was a rumour circulating around his department that for a few years as a graduate student, Church had eaten nothing except a nutrient broth he ordered from a laboratory reagent supplier. He confirmed it with a post on his web page. He got the idea after participating in an experiment on leucine deficiency for which he was forced to subsist on a "semi-synthetic diet" of "cookie-like and jell-O-like 'foods'". "He smelled like yeast extract the whole time," remembers one scientist who knew him then.

People magnet

Church's reputation became a magnet for equally unorthodox students. Robi Mitra, an electrical engineer looking to join the lab in 1997, recalls what Church told him: "He said, 'I like to believe that we get up every day and believe what we do is the most important thing we can be doing, and I'm looking for other people who believe that too'." The pep talk worked: Mitra signed up, and began working on what is Church's current concept for multiplex sequencing, dubbed 'polony' sequencing. In the polony technique, DNA molecules tethered to beads are amplified and processed in discrete units, or 'polymerase colonies' — hence the nickname polony. Separating the DNA into individual colonies makes it much easier to analyse the results of the sequencing reactions.

In 1999, Mitra and Church published the proof of concept that the polony technology worked (R. Mitra & G. M. Church. *Nucleic Acids Res.* 27, e34, 1–6; 1999). Parts of the technology were licensed to the biotechnology company Agencourt in Beverly, Massachusetts. In 2006, Applied Biosystems — the very company that had beat Church's bid to power the human genome project — acquired part part of Agencourt, and took out a licence on Church's polony technology. Last year, Applied Biosystems began selling the 'SOLiD' system — a machine that uses some of the same concepts as Church's sequencing technology. It can sequence the amount of DNA contained in the human genome in less than a month.

The SOLiD is only one of the 'next-generation' sequencers now available, other are made by 454 Life Sciences in Branford, Connecticut, and Illumina in San Diego, California, which have also licensed inventions from Church's



George Church has made his route to work and the letter dismissing him from Duke University available online.

G. CHURCH

lab in the past. So it seems that Church was eventually proven right: a single human genome is old news, and the scientific world is abuzz with projects to sequence more.

In Church's Personal Genome Project, which began last year, his lab will sequence 1% of each participant's genome, mostly protein-coding regions. Church has also sent blood cells from each participant to a New Jersey company that will 'immortalize' the cells, turning them into stable lines for further study. The participants agreed to give extensive information related to appearance and mental and physical health. Last year, they met to discuss questions such as what to tell their families or the press — something that will become an issue when Church begins releasing this information to the world.

Information release

The decision to release data — to participants and the public — is the most controversial aspect. The community norm for genetics studies, and the policy set by funding agencies such as the National Institutes of Health, is that study participants' identities must be kept private. Genetic information may be distributed to researchers, but it must first be 'delinked' from information that could identify its source.

Church calls this a futile and counterproductive policy. As the amount of publicly available information about us increases, so does the likelihood that our identities can be linked to that information — whether or not we want it to be. Meanwhile, scientists can't predict what genetic information will be linked to any trait, so it makes more sense to provide a completely unbiased set of information. "When you walk into a doctor's office you don't have a chador covering your face or a voice-masking device. You walk in freely exposing yourself so that every aspect of your being can be inspected, and that helps physicians make a diagnosis," Church argues. "De-identification creates a research tool that is impoverished relative to what a physician would see."

The difference, however, is that once a doctor makes a diagnosis, the patient controls it, deciding who to tell and what to say. If a person's genetic predisposition to, say, Alzheimer's disease is available to anyone, he or she could face serious consequences — the loss of a job or health insurance, or distressing reactions from friends and family.

Church says that he has already solicited a lot of advice about his project. And he argues that as genomic information becomes more available — as is already happening with the debut of personal genomics services offered by companies such as deCODE Genetics in Reykyavik, Iceland; 23andme (which Church advises) in Mountain View, California; and Navigenics in Redwood Shores, California — people may become less fearful of sharing genetic information with others. If everyone finds that he or she carries some genetic risk, as is likely to be the case, people may not think others' risks are such a big deal.

Scientists can do a lot to minimize the blowback from genetic disclosure. For an example, Church points to the insurance industry. "They're trying to reduce costs, and sometimes they can reduce costs in a way that benefits both the patient and them," Church says. "It's up to researchers and insurance companies and, to some extent, patient advocacy groups, to be creative and figure out where those win–win situations are."

> "If you can't tell me what law of thermodynamics it violates, I don't see why we can't do it."
> — George Church

Church thinks carefully about the societal implications of his work and believes that openness can head off disaster. In 2004, for instance, he helped set up an international effort to head off malevolent uses of synthetic biology. But Church says that there should be restrictions on, or at least monitoring of, technologies that allow individual scientists to synthesize DNA, to minimize the threat that someone could cook up their own deadly microbes.

G. CHURCH

In fact, Church's willingness to engage those outside the scientific community is rare among scientists. It is tempting to speculate that his willingness to deal with outsiders stems from his experience of going against the scientific grain. Although a number of luminaries in the field acknowledge his intelligence and abundance of ideas, several, who did not wish to be quoted for this story, say that they have been annoyed by his stubbornness — his refusal to go along with the 'community consensus', his unwillingness to be swayed from his convictions by other scientists' arguments, and his insistence on pursuing ideas that seem unworkable or impractical. Yet it is also true that courtesy is one of his defining traits. Because he doesn't engage in the sort of public sniping that has marked high-profile endeavours such as the Human Genome Project, many who disagree with Church still have tremendous respect for him.

And Church's critics have to admit, he is remarkably persistent. Few would have pursued a project such as multiplexing for decades after such a discouraging start. Now, Church's determination has spawned a project that is moving genomics out of labs and into regular people's lives.

Church's singular focus is remarkable for someone who has such a broad vision, as he himself admits. Thinking back on his graduate school stunt with homemade blinkers, Church jokes that he has always had a touch of a scientific attention deficit disorder (ADD). "Even back then, I was entirely too broad for my own good," Church says. "But it seems as if society likes my flavour of ADD." ■

Erika Check Hayden writes for *Nature* from San Francisco.

See Editorial, page 745.

Three researchers in Jia Wei's lab with the surname Wang, Xiao-yan, Xiao-rong and Xiao-xue (left to right, with Chinese names above), all publish in English as X. Wang.

Identity crisis

Chinese authors are publishing more and more papers, but are they receiving due credit and recognition for their work? Not if their names get confused along the way. **Jane Qiu** reports.

Jia Wei, associate dean at the pharmacy school of Shanghai Jiao Tong University can remember hundreds of metabolic pathways by heart, but he gets confused by his graduate students' publications. Three of his students — Wang Xiao-yan, Wang Xiao-rong and Wang Xiao-xue (pictured above with Jia) have completely different two-character given names in Chinese, but all publish under the abbreviated name X. Wang. "I really have a hard time sorting out who has published what," Jia sighs.

A similar confusion could arise if John Roberts and Jane Roberts worked in the same lab and both published as J. Roberts. But name recognition in China is compounded by the challenges of transliterating Chinese characters for English-language publications, and by overuse of a few common surnames by the growing population. Estimates by China's Ministry of Public Security suggest that more than 1.1 billion people — around 85% of China's population — share just 129 surnames. Problems with abbreviations, ordering of given names and surnames and inconsistent journal practices heighten the confusion.

When publishing in English-language journals, Chinese researchers adopt a phonetic version of their names, converted through the pinyin romanization system, which uses the Latin alphabet to represent sounds from Chinese. This approach, however, is not bidirectionally unique. There are two Chinese surnames that can be 'spelt' as Wang, for instance. And the problem is compounded by the sheer number of Chinese researchers who have not just the same surname, but also the same initial. Searching the biomedical-literature database PubMed, curated by the US National Library of Medicine, for articles published by 'Wang X' results in 8,904 entries, and this number rises almost daily.

> "I really have a hard time sorting out who has published what."
> — Jia Wei

This issue is not unique to the Chinese. "Japanese and Korean names have the same problem when published in English," says Masao Ito, president of the Human Frontier Science Program based in Strasbourg, France, which promotes international research in the life sciences. Not surprisingly, researchers and editors using search engines and publication databases find it difficult to identify Asian authors. "As a result, Asian researchers are less likely to be invited to participate in collaborative projects or to become reviewers," says Ito.

Publishers make things worse by having varying rules for Asian names. For example, journals differ in how they abbreviate polysyllabic Asian names. If journals abbreviated all the Chinese characters of a given name (Xiao-rong becoming X. R. and Xiao-xue becoming X. X., rather than shortening them to just X.), Jia says that it would help to distinguish between researchers' publications. "This is very problematic when we appraise researchers' performance or during headhunting," he says.

Similarly, some journals insist on listing given names first and surname second, whereas others allow authors to present their names according to the tradition in their own countries. Take, for instance, two researchers previously working on nanocarbon technology at Tsinghua University in Beijing, Yang Wei and Wei Yang (see 'Character confusion'). According to Yang Wei, now at Zhejiang University in Hangzhou, not only did several researchers at Tsinghua

LI HOU-KAI

have exactly the same name as his, but he shares the same initial with several other Yang's working on nanocarbons elsewhere. "You would be lucky to be able to locate the researcher you are looking for," he says.

The problem is sufficiently widespread that some researchers have taken advantage of the ambiguity. Surgeon Liu Hui, who padded his CV with publications by another researcher who shared his surname and initial, rose to become an assistant dean at the prestigious Tsinghua University. But the discrepancies were noticed and he was dismissed by the university in March 2006.

And if Asians can't distinguish between researchers from their own country, it's much more challenging for Westerners, says Gene Sprouse, editor-in-chief of the research journals for the American Physical Society. "When I asked my editors why we have so few Asian reviewers, they said that it's because so many Asian researchers have the same surname and initial that they have difficulties in pinpointing the appropriate ones," Sprouse says. And the problem will only get worse as Asian authors publish more papers, he adds.

Publications from China, Japan and South Korea have increased rapidly in recent years, and by 2006 made up one-fifth of the scientific literature indexed by Thomson Scientific's Science Citation Index (SCI) — roughly two-thirds of the amount from the United States (L. Leydesdorff and C. Wagner *Scientometrics*; in the press). Publications by authors in mainland China indexed by the SCI are growing particularly fast, from 2.3% in 1996 to 8.4% in 2006.

More than words

To address this trend, the American Physical Society has taken the unusual step of offering its authors the option to list their names in Chinese, Japanese or Korean characters, in addition to the transliterated English version. "This is not just a publishing issue," says Sprouse. "A person's name is important. Our initiative is a statement that we respect our Asian colleagues and welcome their submissions to our journals." The society may extend the offer to other languages, such as Arabic.

Although many welcome the society's move, some doubt whether other publishers will rush to adopt the strategy. To print in several foreign alphabets, typesetters would need to incorporate fonts and codes for every one of them, raising costs. Others question how helpful the approach will be on its own. "It would be truly useful only if it's taken up by publication databases so Asian authors could be searched in their own language," says Ito.

These concerns do not yet seem to be troubling literature databases such as PubMed. James Marcetich, head of the index section at the National Library of Medicine, says that he is not aware of problems associated with searching citations with Asian authors. In many cases users can narrow down their search by typing in keywords or the author's affiliation. However, many researchers change affiliations every few years or work in multiple fields. And the narrowing-down process can be daunting and time-consuming.

"It's all about efficiency," says Sun Xiao-peng, a manager at the Beijing office of the Dutch science and technical publisher Elsevier. "It's a matter of whether an author can be located in 15 minutes or 6 hours." He doubts whether searching in native languages would help because it's extremely difficult for researchers to search names in a language unfamiliar to them. He says that Elsevier has tools — including its Scopus author identifier — that provide an alternative way to identify both Asian and Western authors with common surnames.

> "You would be lucky to be able to locate the researcher you are looking for."
> — Yang Wei

Launched in 2006, the author identifier assigns a unique number to the roughly 20 million authors who have published articles in the 15,000 journals covered by the Scopus database. An algorithm distinguishes those with similar or identical names on the basis of their affiliations, publication history, subject areas and co-authors. Scopus claims to have achieved 99% certainty for 95% of its records, and designates a webpage to each author so that they have an overview of their personal data and can make corrections. "This is akin to a researcher's passport, which follows them wherever they go," says Sun.

Elsevier is not the only company offering such commercial products. Last month, Thomson Scientific in Philadelphia unveiled its own ResearcherID, which allows researchers to create stable personal identifiers to manage their citation metrics. The software allocates a number during a one-time registration. "ResearcherID resolves any ambiguity surrounding published works and provides a safe space for virtual collaboration," says Jim Pringle, Thomson's vice-president of product development. The service currently has some 3,500 invited users.

United front

Librarian Susan Fingerman at Johns Hopkins University's Applied Physics Laboratory in Laurel, Maryland, thinks that Elsevier's claim of accuracy in identifying authors "is probably overblown". In any case, she argues, these approaches can provide only part of the solution because the problem of author identification lies farther upstream in publishing — with journals having inconsistent rules for Asian names. She thinks that publishers should get together to agree on a uniform approach to author names. "Only then could author identification systems be truly useful," she says.

The products currently in the market, however, fall short of providing an identifier that works across all databases. One solution may come from CrossRef — a coalition of 2,046 scholarly publishers, including Elsevier and Nature Publishing Group — that wants to introduce a Contributor ID, similar to its digital object identifiers (DOIs) for electronic content. "CrossRef has been considering the idea of a universal author DOI registration for sometime," says Geoffrey Bilder, CrossRef's director of strategic initiatives. Bilder says that both algorithm and user-generated systems are prone to errors and what CrossRef can offer is authentication, which is essential if an identifier is to be used for professional purposes.

Later on this year, CrossRef will trial a prototype system that will invite contributors — including authors of books and academic papers — to register their material. The publications listed by the authors will then be authenticated through linking to publishers' websites.

"A universal author-identification system could help to clarify the confusing situation," says Wang Xiao-yan. There are some 93 million people in China who share the surname Wang, and so the chances of her working alongside, or in the same field, as another X. Wang are high. She worries that misidentification issues could prevent her from competing with her Western colleagues on an equal footing. "This could be a stumbling block to my career," she says. ■

Jane Qiu writes for *Nature* from Beijing.

Character confusion

Apart from unhelpful abbreviations (see image opposite), there are two other ways in which conversion of Chinese names into English can cause problems.

Li Yan Li Yan

Phonetic confusion Two researchers with different Chinese names both have their names transliterated as Li Yan in English.

Wei Yang Yang Wei

Mixed-up order Researchers named Yang Wei and Wei Yang can be confused if journals have different rules for the ordering of given names and surnames.

Students find out that bacteria can cheat too

SIR — I shared S. P. Diggle and colleagues' Letter 'Cooperation and conflict in quorum-sensing bacterial populations' (*Nature* **450**, 411–414; 2007) with my ninth-grade mathematics students, because I was fascinated by the bacterial communication system known as 'quorum sensing' — as well as by exploitation in the prokaryotic realm.

I explained that even bacteria know how to assess their own numbers, which enables them to coordinate their behaviour and survival. The importance of mathematics and evidence of cheating in the world of bacteria could be compared with the case of a student who exploits the cooperative calculation of an answer to a specific algebraic problem set during, and resulting from, group work.

The discussion generated an opportunity for the students to identify certain similarities between bacterial behaviour and the dynamics of more complex organisms such as humans, and to show that mathematics is as ubiquitous as bacteria.

I have received positive feedback from parents encouraging the continued integration in the classroom of mathematics with other scientific disciplines and real-world scenarios. Thanks to the authors and to *Nature* for publishing a paper understandable to the average reader.

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European adventures did nothing for people of Egypt

SIR — Napoleon's imperial project in Egypt was far from being romantic, as the headline on the Book Review of Nina Burleigh's *Mirage: Napolean's scientists and the unveiling of Egypt* would suggest ('The ultimate Romantic adventure' *Nature* **450**, 793; 2007). Like other acts of imperialism, it was oppressive and demeaning to the lands and cultures that it claimed to liberate, study or civilize.

Did the learned men who were part of Napolean's imperial adventures ever wonder whether the often-brutal manipulation and subjugation of another civilization was in keeping with the highest ideals of the Enlightenment? Did any real benefit accrue to the peoples of Egypt or India when their treasures were revealed to Europe?

These wonders had been understood, interpreted and revered by the local populations within the context of their own culture and history long before Europeans came across them. Their 'discovery' by Europeans largely paved the way for further exploitation. They filled the coffers of the colonial masters, but often impoverished the colonies. Looked at in isolation from the fundamentally unjust social and political policies that sustained them, the scientific achievements seem to be stunning. But any honest evaluation of the scientific by-products of imperial projects is incomplete without acknowledgement of the exploitative nature of imperialism.

Ninad Bondre
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Funding cuts leave 'golden era' looking tarnished

SIR — Last May you ran an Editorial headlined 'Never had it so good?' (*Nature* **447**, 231; 2007). "The Blair–Brown era has been a golden one for British science," it said. I found this surprising, as the nadir for R&D funding (as a proportion of gross domestic product) over the past three decades occurred under the Blair government. Funding fell from 2.38% in 1981 to an average of 1.85% between 1997 (when Blair came to power) and 2003, according to the Organisation for Economic Co-operation and Development (OECD) *Factbook 2006*. UK funding remains well below the average, both in the 27 countries of the European Union and the 30 countries in the OECD.

I wasn't moved to write at that time, but the recent announcement by the UK Science and Technology Facilities Council of an £80-million (US$156-million) shortfall in its budget (see *Nature* **450**, 1127–1128; 2007) has made me change my mind. This is going to translate into a (minimum) 25% decrease in grants for the next three years, with no prospect of improvement after that — a situation that will be catastrophic for physics, astronomy and planetary science in the United Kingdom, and for the morale of those research communities. As a UK planetary scientist, this does not feel like a golden era.

Phil Bland
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Future stars of the lab need realistic role models

SIR — The Futures story 'When Britney Spears comes to my lab' (*Nature* **451**, 106; 2008) was written tongue in cheek, but I am disappointed that *Nature* published it. Those of us who have decided "to put in more hours at the lab for the same reason all good scientists do" will resent being compared to even a fictional Ms Spears. Your article pokes fun at the pop singer, but it also indirectly undermines the dedication of real young women starting out on a research career.

The satirical Spears is helped in her scholarly endeavours by her pop-singer's salary, which exceeds that of a real graduate student by several orders of magnitude. Those who, like Spears, are parents must struggle on their stipends and balance the demands of scientific research with those of their children — not a problem for the wealthy Spears of academic fantasy.

Her frivolous appearance is also caricatured in the laboratory setting, reflecting an attitude that can still influence how women scientists dress. She also seems untroubled by the prospect of a fight for a place in the sciences. But that requires more than just inspiration: add quantities of caffeine, a fear of failure and years of hard work and personal sacrifice. I have yet to embark on the next step after graduate school, but I can only hope to have a fraction of the impact on society that the Dr Spears of Futures has on diabetes. I'll try to work harder.

I do agree, though, that we must find more ways to inspire upcoming generations to pursue endeavours that will benefit humanity, instead of glorifying reality television and tabloid superstardom.

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Freeing more carbon will accelerate global warming

SIR — Although the Letter by D. M. Jones and colleagues, 'Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs' (*Nature* **451**, 176–180; 2008), describes fascinating science, the progression of this research to a viable technology would represent a dangerous advance in humankind's ability to accelerate global warming.

The prospect of increasing the energy efficiency of hydrocarbon extraction from tar sands, as referred to in the Letter, is highly questionable. Any technology that will increase our capacity to free surface or sub-surface carbon is surely undesirable, and will reduce the imperative to migrate to non-carbon or closed-carbon energy cycles.

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Contributions to this page may be submitted to correspondence@nature.com. We welcome comments on publishing issues at Nautilus (http://blogs.nature.com/nautilus).

BOOKS & ARTS

REUTERS

A Palestinian confronts an Israeli policeman, but 'psychological' barriers make violent behaviour infrequent.

A close look at conflict

Psychology, neuroscience and physiology are missing from a new sociological study of violence.

Violence: A Micro-Sociological Theory
by Randall Collins
Princeton University Press: 2008. 584 pp. $45.00, £26.95

Margo Wilson & Martin Daly

Violence matters. It has been scrutinized by numerous researchers, from anthropologists to zoologists. Yet virtually all the discoveries and insights so accumulated — including those from sociology — get short shrift in this ambitious book. Author Randall Collins discusses "at least 30 types of violence", from bullying and domestic violence, through staged fair fights and contract killings, to gang fights, sporting brawls, riots, police brutality and warfare. He draws on a vast opportunity sample of photographs, documentary films, and participant and witness accounts. Nevertheless, his agenda is tightly focused. He gives all these phenomena the same two-component 'micro-sociological' analysis.

First, Collins describes the action in some detail — facial expressions, body postures, approaches and retreats, the brandishing and use of weapons. It is refreshing that he should tout the value of watching one's study animal, for with a few notable exceptions, such as Erving Goffman, sociologists have evidently done too little of this. The ethological study of confrontation and aggression has a long and fruitful history in non-human animals and human beings, especially children. Statistical studies (using sequential analysis) of social interactions are well developed and have yielded significant results. The risks of subjectivity and arbitrariness have been countered by careful quantification and by assessing inter-observer reliability. Collins's descriptive agenda would benefit from attention to the efforts of these predecessors.

The second component of Collins's 'micro-sociological' analysis is an attempt to explain what is happening in terms of inferred emotional states and processes. The hypothesized emotional processes — 'entrainment', 'interaction ritual chains', 'forward panic' — are central to the book's theory and are apparently original. Unfortunately, Collins does not define them sharply, nor does he make an explicit case for their validity.

One big theme of this book is that "violence is hard". Collins makes a persuasive case for there being psychological barriers to violent behaviour that make it infrequent, inefficient and emotionally fraught. For example, he reviews at some length the substantial evidence that only a minority of soldiers fire their weapons or otherwise strike in combat situations, and that even fewer do so effectively. Similarly, in street riots or demonstrations, only a small minority are actively violent.

However, Collins creates a straw man when he asserts that all existing explanations "assume violence is easy once the motivation exists". This is a fair criticism of Hollywood portrayals, but not of the scientific literature, in which the infrequency of damaging violence has been much remarked on in studies of both human

and non-human conflict. Collins rightly stresses that confrontations seldom progress beyond threat and bluster, and he is right to ask why. His answer is, at best, shallowly descriptive: "No matter how motivated someone may be, if the situation does not unfold so that confrontational tension/fear is overcome, violence will not proceed." Factors that allegedly facilitate overcoming this "tension/fear" are the expressions, postures and behaviour of the other party, numerical or technical advantage and the support of an audience.

Again, much of this is not news. Threat displays, bluffing and audience effects on violence all have literatures of their own. It is 35 years since John Maynard Smith and George R. Price provided, in these pages (*Nature* **246**, 15–18; 1973), a game theoretical analysis of why threat displays are common and damaging violence is rare. Posturing conveys information about both fighting ability and the individual-specific value of winning a contest. Violence is "hard" because it endangers the perpetrator as well as his target.

Sociologists often defend the autonomy and importance of their discipline by disparaging 'psychologizing'. To his credit, Collins realizes that phenomena such as emotions are essential components of a satisfactory explanation of human behaviour. Yet, perhaps to retain his sociological 'cred', he eschews consideration of current knowledge in psychology, physiology and neuroscience. He ignores a vast body of emotion research with direct implications for his theory, and much highly relevant work on the relative importance of situational versus individual attributes in the variability of behaviour. Moreover, Collins resurrects the notion that motivation is a sort of 'energy' that can be dammed up only to spill over into irrelevant activities, without acknowledging the devastating critiques by Robert Hinde and others that led to the rejection of such Freudian/Lorenzian models in the 1960s. Collins's goal of characterizing the micro-dynamics of social interaction is laudable, but he is blinkered by disciplinary parochialism.

Does Collins really have the 'theory' his subtitle promises? If so, it is not one from which any testable hypotheses flow — theory here seems closer to its meaning in the humanities than in the sciences. Reading the book in this spirit, one finds keen observations and persuasive interpretations of aspects of police violence, domestic abuse, gang fights and the behaviour of men at war. But the road to a scientific understanding of violence is interdisciplinary. ■

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The invisible revolution

Nanosciences: La Révolution Invisible
By Christian Joachim and Laurence Plévert
Seuil: 2008. 182 pp. Can$31.95, €18
(in French)

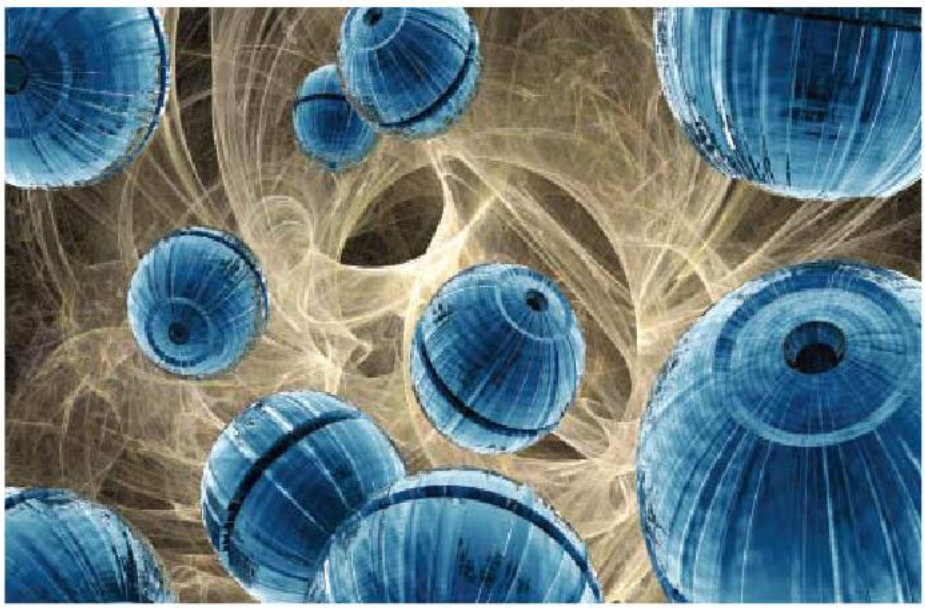
Vincent Dusastre

Nanotechnology was originally defined in the 1970s as the science of manipulating atoms and single molecules. Its remit has since expanded to embrace all technologies capable of building structures at the scale of a billionth of a metre. How and why did this change happen and has it affected the field's development?

Politics and big business caused the shift, according to physicist Christian Joachim and journalist Laurence Plévert. In their French-language book they argue convincingly that this more inclusive definition has also altered the initial goals of nanotechnology research.

Richard Feynman's 1959 lecture, 'There's Plenty of Room at the Bottom', touched on the difficulties of controlling matter at the atomic scale. But the term nanotechnology was first coined by Norio Taniguchi, at Tokyo Science University, in 1974. This was about the same time that Ari Aviram and Mark Ratner, at IBM, proposed the idea of a single-molecule rectifier.

In the early 1980s, the invention of the scanning tunnelling microscope, which could image and manipulate single atoms, made it possible to design nanoscale machines from individual atoms and molecules. Joachim, who worked as a young researcher at IBM at that time, takes us on a nostalgic personal journey from single molecules to today's molecular machines, such as organic molecules capable of moving and performing calculations. Given the visual interest of the tiny machines, it is a pity that his gripping story is not illustrated.



LAGUNA DESIGN/SCIENCE PHOTO LIBRARY

Molecular-scale machines could one day have medical applications such as removing cancerous cells.

Until the 1990s atomic manipulations were developed with sustainability in mind. Hope was that building machines from the bottom up, atom by atom, rather than top down, etching them from larger blocks, would minimize the energy and materials expended in manufacturing. US industrial lobbies then broadened the definition as a way of accessing public funds earmarked for materials and chemistry research and development. These lobbies convinced the Clinton administration to launch the National Nanotechnology Initiative in 1999, which fostered energy-intensive top-down techniques for fabricating and sculpting objects less than a micrometre in size.

The initiative's generous funding boosted industrial development and innovation in globally competitive areas such as microelectronics and biotechnology. For fear of being left behind, funding agencies worldwide, including in Europe and Japan, quickly adopted similarly broad definitions. More recently, nanotechnology has ventured even further. It is now portrayed as a key and novel way of tackling the world energy crisis and water shortage. Yet top-down production is intrinsically wasteful of materials and energy.

The drive to miniaturize has been with us a long time. Joachim and Plévert chart the progression from ancient Greek astrological clocks to James Watt's steam engine to the discovery of the electron and finally today's microelectronics. The authors explain clearly that, when

EXHIBITION

Essence of creation

Isabelle Kaufmann

Both biotechnologists and artists create. *Genesis — The Art of Creation*, at the Zentrum Paul Klee in Bern, Switzerland, suggests their methods and aesthetics show unexpected kinships.

The Zentrum Paul Klee houses the largest collection of works by the eponymous early-twentieth-century painter, who spent much of his life in Bern. Working at the interface of figurative and abstract art, Klee studied the forms of plants, shells and stones, and drew from them new and imaginative shapes.

Genesis — the process of creation — was a key theme. According to Klee, the painter starts with the basic elements of point, line, tone and colour (pictured, *physiognomische Genesis*, 1929). He experimented with them, recombining them, and so bringing something new into existence. Replace brush and canvas with pipette and test tube, and this, the exhibition posits, could be a genetic engineer rearranging DNA and creating new forms of life.

Klee's geometric compositions and chimaeric beasts are juxtaposed with paintings by fellow modernists such as Piet Mondrian and anatomical drawings by Leonardo da Vinci. Exhibits by contemporary artists borrow the techniques of biotechnology. A video of transgenic organisms in Eduardo Kac's installation projects a plate of bacteria expressing blue or yellow fluorescent proteins; as the cells grow, mutate and conjugate, new colour variations emerge.

Also on display are paintings and prints inspired by microscopic images. Ross Bleckner's *In Replication* imagines the scene inside a dividing cell: a wild and colourful dance of molecules pairing, entwining and separating. David Fried's bubble shapes recall pictures of fertilized egg cells, captured in reproduction, and growing in harmonic patterns. These pieces demonstrate that scientific images enrich our knowledge and that their unusual beauty has a truth of its own. ■

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***Genesis — The Art of Creation* (until 27 April) is at the Zentrum Paul Klee, Bern, Switzerland (www.zpk.org).**

PRIVATBESITZ SCHWEIZ, DEPOSITUM IM ZENTRUM PAUL KLEE, BERN

applied to integrated electronics and electromechanical systems, lithographic techniques are reaching a physical limit. Moreover, below a scale of tens of nanometres, fundamental problems such as interconnection and quantum effects arise.

Today, nanotechnology is embracing biology. The authors rightly dismiss fantastic worries that our DNA may be modified by nanobots capable of getting into cells as well as nanotechnology's dubious association with genetically modified organisms. But they are rash to focus on recent controversial observations of bacteria less than 100 nanometres long that might be incorporated into molecular machines. They ought instead to have emphasized current research efforts to build machines from self-assembly and supramolecular chemistry.

Caveats aside, this popular book sets out the science that underpins nanotechnology and in so doing gives a realistic picture of its impact, applications and political, economic and societal context. ■

Vincent Dusastre is editor of *Nature Materials* (www.nature.com/naturematerials).

Genomes evolve, but how?

The Origins of Genome Architecture
By Michael Lynch
Sinauer: 2007. 510 pp. $59.95 (hbk)

Axel Meyer

"Nothing in biology makes sense, except in the light of evolution," said the great geneticist and evolutionary biologist Theodosius Dobzhansky. Twenty-five years on, genomics as a discipline has yet to embrace evolution fully. Michael Lynch is an exception. His timely textbook demands that population thinking, population genetics and evolutionary theory be meshed more explicitly. After all, genomes did not appear suddenly from nowhere, and mutational changes from single base-pair substitutions to whole-genome duplications are at least one basis of molecular as well as phenotypic evolutionary change.

As the cost of genome-sequencing falls and more genomes of the major model systems are sequenced, evolutionary biologists have more say in which organisms will be investigated next. Population samples of, for example, the model species *Drosophila* (fruitflies) are a good target.

Yet this line of research is still driven strongly by technical innovation, such as the speed and cost of data collection, rather than the testing of theories that might direct future experiments. Genomics research is progressing incredibly fast, off the back of genomic data that are being produced ever more rapidly. Still in a stage of wondrous discovery, this nascent field today evokes the excitement of the early days of natural history.

Lynch is a population geneticist who has made major contributions to numerous evolutionary questions and recently expanded his interests to genomics. He has published landmark studies on mutation rates, gene

duplication and the functional diversification of genes.

In *The Origins of Genome Architecture*, he advocates using population genetics to understand genomes because the mechanisms involved can explain changes in gene frequency across generations and elucidate genome evolution. For Lynch, population genetics and some non-adaptive mechanisms in particular suffice to understand genomic evolution. He argues that invoking 'mythical macroevolutionary forces' is unnecessary.

Almost every page introduces interesting, unanswered problems — a goldmine for students in search of a thesis topic.

Lynch goes a step further by combining molecular mechanisms and evolutionary theory into a coherent evolutionary genomics framework and claiming it as the next phase of evolutionary biology. The ability to straddle both disciplines is rare and hardly attempted in the other direction — few molecular biologists know much about evolutionary biology. Rightly, Lynch laments this asymmetry.

This book is a must-read for every genome researcher; evolutionary biologists will also profit. It reviews and analyses, competently and thoroughly, a huge range of topics, from the origin of eukaryotes to sex chromosomes. It is the best, most up-to-date and thorough summary of genome evolution published. Arguments, hypotheses and supporting data are presented clearly and cross-referenced. Only the most necessary equations interrupt the flow. Almost every page introduces interesting, unanswered problems, making it a goldmine for graduate students in search of a thesis topic. Rarely have I scribbled so many pencil marks in a book's margins.

The last chapter, distinctively entitled "Genomfart" (meaning 'place of passage' or 'the way forward' in Swedish), discusses how much scientific meat lies behind fashionable buzzwords such as complexity, modularity, robustness and evolvability. It alone provides enough intellectual fodder for a stimulating seminar series. Not every evolutionary biologist, genome researcher or 'evo-devo-ist' will agree with Lynch's strong opinions that largely non-adaptive forces shaped genomes, but it is a debate worth having.

As long as we remain unsure what a gene is, we are a long way from understanding genome evolution. That so much is still unknown should not worry us. Rather, it should reassure the next generation of evolutionary genomic biologists that there is much to be discovered. ■

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FESTIVAL

Neural networking in Manhattan

Giovanni Frazzetto

New York city will be criss-crossed this spring by a net of brainy ideas. More than a hundred public events will link neuroscience with art, music and meditation in the city's Brainwave festival, which runs until June.

The metropolitan mix gives the festival its peculiar flavour. Musician Lou Reed introduces and discusses his latest compositions about meditation. Neuroscientist Joseph LeDoux examines sources of fear, and asks how Buddhist practitioners seek to master this emotion, before dashing off to play guitar in his band, The Amygdaloids.

The festival's contemporary art show, *Brainwave: Common Senses*, opens this month at the cultural centre Exit Art. Cleverly curated, it features images inspired by brain anatomy and function, as well as representations of aspects of consciousness, cognition and memory. The works on show address new technologies of neuroscience and the joint outputs of artists and scientists who have puzzled together over the workings of the brain.

Suzanne Anker extrapolates neurological processes from images of Rorschach tests and brain scans, and renders them into three-dimensional sculptures that are suggestive of bones, sea creatures and body parts. Levels of cognition and perception are represented in a multisensory and interactive installation by artist collective SERU.

Devorah Sperber's apparently random arrangement of 875 spools of coloured thread (pictured) coalesces into a replica of Leonardo Da Vinci's *Mona Lisa* when observed through a small sphere that mimics the human eye.

All this attention may be symptomatic of the rise of a 'neuroculture', in which neuroscientific understanding becomes part of our daily life. However, some fear that if we gain too much scientific knowledge about how the brain accomplishes creative tasks or causes emotions then it will lead to disenchantment. Brainwave seeks to show that this need not be the case. ■

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Brainwave festival events run across New York City until June (www.brainwavenyc.org). *Brainwave: Common Senses* runs from 16 February to 19 April at Exit Art, New York city (www.exitart.org).

NEWS & VIEWS

C. BOISVIEUX/CORBIS

Figure 1 | Desert storm — Wadi Mur, Yemen, 2003.

GRANULAR MATTER

Static in motion

Troy Shinbrot and Hans J. Herrmann

Wind-blown desert sands can charge up spontaneously. But although sand flow and the forces on charged bodies are well studied separately, surprisingly little is known of what happens when the two combine.

The strange things that happen when granular media and electrostatic charge mix have long exercised the minds of both scientists and engineers. In the latest episode in this saga, Kok and Renno, writing in *Physical Review Letters*[1], focus on how static charges affect the aeolian — wind-borne — transport of sand. As the basic ingredient of sand encroachment and dune motion (Fig. 1), this is a process of more than academic interest, with implications for desertification and coastal management.

But first some history. As long ago as 1867, William Thomson, better known as Lord Kelvin, studied the charging of both falling grains and water droplets in his attempts to understand the origin of atmospheric lightning[2]. Eighty years later, physicist E. W. B. Gill drew on his observations of sparking and radio interference on the Macedonian front during the First World War to produce a similar effect in the laboratory[3].

Grains in an electric field are also known to self-assemble into complex patterns[4]. Other poorly understood, but bothersome, phenomena can be traced back to particle charging, too. Clouds of charged dust regularly produce devastating explosions in grain and coal plants[5]. On the Moon, charged grit attaches itself to spacesuits and works its way into suit joints, causing them to leak air and so cut exploration time. The problem was such that Apollo 16 commander John Young considered dust the number one concern in returning there. That sentiment was echoed in NASA's most recent study on Mars exploration[6].

Back on Earth, aeolian flows have been measured in both field[7] and laboratory[8] experiments. These investigations showed that charges acquired by wind-blown sand can be sufficient to levitate the grains, and even to violently eject them (Fig. 2, overleaf). Despite experimental data demonstrating that grains readily acquire substantial charges, with few notable exceptions[8], surprisingly little fundamental analysis has been done on how charge affects granular flow, and several essential questions remain to be answered.

First, it has been established that even carefully prepared, identical materials charge one another[9,10]. But how exactly does this work? In a desert environment, where wind-blown sand grains have little but other, similar grains to rub against, yet still manage to acquire charge, this is a significant puzzle. Kok and Renno[1] go some way to finding an answer. They have constructed an effective charging relation, for pairs of particles of the same composition but different sizes, that describes the empirical fact that smaller sand grains tend to charge negatively, and larger grains positively.

Even so, the mechanism for the underlying charge transfer remains at best tenuously understood. One proposal is that smaller particles rise to the top of a sand cloud, where they encounter more highly mobile, negative ions in the air, and so themselves become negatively charged[1]. Another theory holds that asymmetric particle collisions heat the smaller particles more effectively than the larger ones, possibly leading to a transfer of charge between them[10].

A second unanswered question is how the charge that is measured on sand grains affects aeolian flow. Kok and Renno produce a model for the motion of wind-blown sand ('saltation') that takes electrostatic interactions into account by assuming charged sand grains to be attracted to Earth, which acts as an infinitely large, oppositely charged particle. This

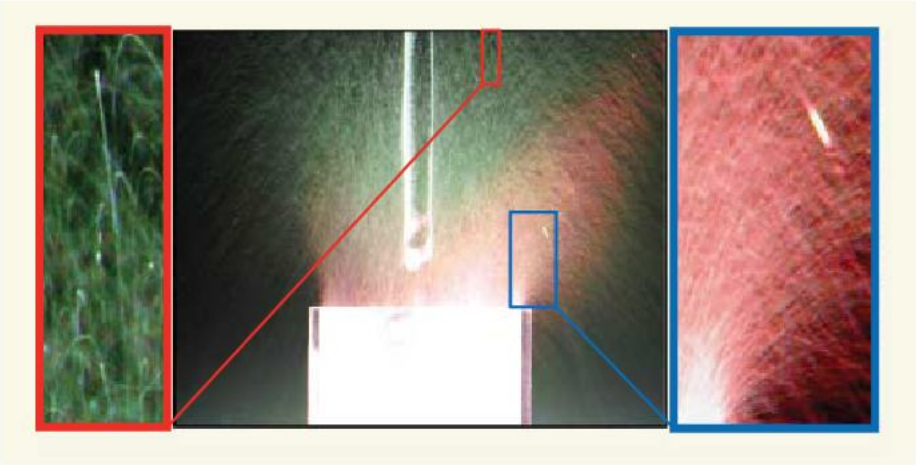

Figure 2 | Granular fountain. In this laboratory demonstration (centre) of the explosive potential of charged grains, glass beads 500 micrometres in diameter are charged by repeatedly pouring them through a vertical acrylic tube into an acrylic container. The charges on the grains become so large that the beads cannot remain at rest, and they spontaneously form a fountain that erupts from the container, even after the inflow has ceased. If the tube is electrically grounded, these ejections do not occur. Left inset, trajectories of individual grains; right inset, an apparent example of a granular aggregate coexisting alongside individual grains. Kok and Renno[1] produced a model of the development of such charge on sand. (Colours are digitally enhanced; image and experiment courtesy of F. K. Wittel, ETH-Zürich.)

causes charged sand grains to fall back down faster than they would do otherwise. At the same time, the authors note that charged grains tend to levitate (or even jump considerable distances, as seen in Figure 2), and so should become more easily airborne than neutral grains. Both of these apparently contradictory propositions seem sound, but their combined effect on sand flow, and the effect of charges of both signs on a saltating bed, remain to be fully understood. Further progress will require a concerted combination of careful measurements and simulations rarely attempted to date[8].

One final, extremely basic, question is: will charged sand grains attract or repel each other? Both phenomena are observed. If grains simply acquire a uniform charge, they will of course repel each other according to Coulomb's law. But dipole moments — a result of an uneven distribution of electric charge — on single particles have also been observed, and local assemblies of charge are known to form at points of contact between grains[11]. Consequently, similarly treated grains are seen to both repel each other and to adhere in clusters[3] in the same system (Fig. 2). How a collection of grains in, say, a desert sandstorm will behave will ultimately depend on the charge distributions on individual grains, which are poorly understood.

It is remarkable that, since Kelvin's time, so basic a question as how a common material such as sand becomes charged and flows remains unresolved. Work such as that of Kok and Renno[1] demonstrates how complex the research challenges are. The simple combination of grains and charge will no doubt be generating more surprises in the years to come. ■

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EVOLUTIONARY BIOLOGY

A first for bats

John Speakman

Which came first as bats evolved — flight or echolocation? Newly described fossils favour the flight-first hypothesis. But these creatures may have been otherwise equipped for flying at night.

A long-standing debate about the processes that led to the evolution of modern bats takes a new twist with the discovery of remarkable fossil bats recovered from the Green River formation in Wyoming. The fossils, which constitute a new genus and species, are described by Simmons *et al.* on page 818 of this issue[1]. Phylogenetic analysis and comparison with other fossil bats recovered from the same formation, and from the Messel formation in Germany, indicate that this is the most ancient species of bat yet discovered.

The problem of understanding bat evolution dates back at least to Charles Darwin, who in *The Origin of Species* enumerated a list of difficulties he saw with the theory of evolution by natural selection. The example often discussed is the origin of the eye. But Darwin also mentioned the vexed issue of how bats had arisen from terrestrial ancestors. The discovery of echolocation in bats about 50 years ago[2] added an additional feature to the conundrum of the early evolution of bats. This currently boils down to one big question: which came first, echolocation or flight[3,4]?

For a long time, 'echolocation first' held sway. Ancestral 'pre-bats' were hypothesized to have been small terrestrial or arboreal echolocators that detected passing insects using their echolocation and snatched them from the air[4]. This favoured the extension of the arms and digits to facilitate prey capture, perhaps with webbing between the digits. Eventually, these animals started leaping out to capture insects, using their echolocation to guide them to a landing spot and their extended arms and digits as an aerofoil. From this point they started hunting from perches (known as perch hunting) and eventually developed fully powered flight (called aerial hawking; Fig. 1).

Supporters of the echolocation-first hypothesis pointed to the existence of terrestrial animals, such as certain shrews, that have rudimentary echolocation systems; and to the fact that the most primitive extant bats often use perch hunting, and lack a feature known as the calcar, which is also absent in the most ancient fossil bats. (The calcar is a cartilaginous spur projecting from the base of the lower limb and running along the edge of the membrane between the hind limbs and tail.) Moreover, the idea that bats might have evolved the ability to fly before they could orient themselves in darkness was seen as highly unlikely.

However, around the end of the 1980s, evidence accumulated, including work from my own group, that favoured the 'flight-first' hypothesis. One paper[5] showed that, for a bat hanging at rest, echolocation is extremely energetically costly. This high cost probably explains why no terrestrial mammals have evolved full-blown echolocation systems such as those used


M. DURHAM/MINDEN PICTURES/FLPA

Figure 1 | In flight today. This is a little brown bat, an aerial hawker, pictured flying at night in the Rogue River National Forest, Oregon.

by bats. However, a second paper[6] showed that when a bat takes flight these costs disappear. This is because of a remarkable coupling of the beating of the wings with the ventilation of the lungs and production of the echolocation pulses[7]. When a bat hangs stationary and echolocates, it must contract its muscles specifically to generate a forceful expiratory burst, and this is where the large costs come from. When a bat is flying, it is already contracting these muscles, so in effect echolocation when flying is free (or at least substantially cheaper).

But what about the problem of bats flying in darkness before they could orient themselves? A hypothesis I favour[8] is that the earliest ancestors of bats may have been diurnal, and had visual means of orientation — but were perhaps forced to become nocturnal by the appearance of avian predators, shortly after the dinosaurs became extinct around 65 million years ago. Some then evolved echolocation, whereas others became nocturnal vision specialists.

Until the discovery of the specimens reported by Simmons *et al.*[1], the fossil record has been rather unhelpful in resolving these issues: the earliest-known bats, which have been recovered from Eocene deposits around 50 million years old, are fully formed bats very similar to extant ones[9,10]. It has been possible to show that these bats were all already capable of echolocation by examining the size of the cochleae in their ears; cochleae are massively enlarged in echolocators. Previously described fossil bats were all already capable of both flight and echolocation[11,12].

The bat described by Simmons *et al.*[1] is represented by two fossils dating to about 52.5 million years ago; one is shown on the cover of this issue. The bat's wing morphology is very similar to that of extant species, except that it has claws on its digits. But in all other respects this is clearly a bat capable of powered flight. Unlike other primitive fossil bats, this species also has a calcar — indicating that the absence of a calcar and perhaps therefore perch hunting are not ancestral traits. But the real insight provided by this fossil is the spectacular finding that it does not have enlarged cochleae. By inference, therefore, it was not capable of echolocation, providing the first direct evidence supporting the flight-first hypothesis. Examination of the bat's limb proportions suggests that it was probably arboreal, as has generally been assumed by proponents of both echolocation-first and flight-first hypotheses.

A remaining question is whether this bat was nocturnal or diurnal. Examination of the size of the eye sockets might help, as nocturnal non-echolocating animals generally have enlarged eyes and so enlarged eye sockets. Unfortunately, the two specimens described by Simmons *et al.* cannot answer this question, because their upper skulls are crushed and their eye sockets cannot be reconstructed. Perhaps that was too much to hope for. As it is, these outstanding fossils considerably advance our understanding of bat evolution. ■

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ASTROPHYSICS

A story of singular degeneracy

Edward P. J. van den Heuvel

Astronomers have a choice of two models of how type Ia supernovae arise. The progenitor for one of these huge stellar explosions has now been discovered, bringing a definitive judgement a little closer.

On page 802 of this issue, Voss and Nelemans[1] report that they have found a luminous source of low-energy X-rays at just the position where, on 5 November last year, the type Ia supernova SN2007on was sighted. The discovery, made in archive data from NASA's Chandra X-ray-observatory satellite, is the first-ever detection of the progenitor of a supernova of this class. It lends support to one particular model for their origin: the 'single degenerate' model, in which the transfer of mass from a normal hydrogen-burning star to an exhausted white-dwarf star triggers the white dwarf to explode.

Type Ia supernovae are crucial objects for cosmology. Because the evolution of their luminosity curves is precisely known, their maximum measured luminosity can be used to assess their distance from us, making them useful 'standard candles' for measuring the expansion history of the Universe. Such measurements led in 1998 to the discovery that the expansion of the Universe is accelerating[2–4]. This phenomenon was ascribed to a repulsive force deriving from a newly proposed type of vacuum energy ('dark energy') that makes up some 70% of the total mass–energy budget of the Universe.

In the spectra of type Ia supernovae, hydrogen and helium are conspicuously absent, whereas spectral lines emanating from silicon, calcium and iron are present. From this observation, combined with the uniform nature of these objects' luminosity curves, astronomers

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50 & 100 YEARS AGO

50 YEARS AGO

Women who have had a university education want to use their capacities to the full and to make a contribution to society. How can they combine this with marriage and motherhood? Mrs. Judith Hubback, herself a graduate mother, tried to find this out by sending out, in 1953, a questionnaire to 2,000 married women graduates ... It is found that the marriage rate for women graduates is almost up to the normal, that they tend to be more fertile than average, that it takes some fifteen years before the last child is at school, that circumstances during the period of raising children give little opportunity for systematic intellectual pursuits or for an outside career, but that part-time teaching is the career most easily combined with family life ... A whole chapter is devoted to overtiredness, due to diffuse, routine domestic work, which leads to frustration, and it is found that work outside the home, even part-time, results in a freshness of outlook and not in extra fatigue. In the discussion, the need is stressed for a sane compromise between the biological aspects of a woman's life, as a mother carrying on the race, and the intellectual side developed by a university education.

From *Nature* 15 February 1958.

100 YEARS AGO

Prof. Dunbar, as the result of a series of experiments conducted over a long period and with every care, has come to the conclusion that the bacteria are not an independent group of organisms, but, together with some of the yeasts and moulds, are stages in the life-history of green algae ... A pure culture of a single-celled alga belonging to the Palmellacia was obtained, but by modifying the culture medium by the addition of acid, alkali, or traces of copper salts, other organisms, generally bacteria, occasionally moulds and yeasts, and even spirochaetes, made their appearance in the pure cultures.

From *Nature* 13 February 1908.

conclude that type Ia supernovae are explosions of 'electron-degenerate' stars consisting of carbon and oxygen. In these 'dead' stars, known as CO white dwarfs[5], the outward pressures caused by the processes of nuclear fusion at some point ceased to provide a counterbalance to the star's own gravity. The star's matter collapsed down so far that all its electrons sought to squeeze into their lowest energy state (to become 'degenerate'). At this point, the Pauli exclusion principle — which states bluntly that this can't happen — put a brake on the gravitational collapse.

If, however, the mass of a white dwarf increases above a certain limit — the Chandrasekhar limit, about 1.39 times the mass of the Sun — the degeneracy pressure created by the exclusion principle cannot prevent further collapse. The resulting rapid increase in density and temperature causes the runaway fusion of carbon and oxygen nuclei to heavier elements, ending mainly in iron. The sudden and enormous energy release of this chain reaction blows up the whole star.

These thermonuclear supernovae are very different from their cousins, the core-collapse supernovae. Core-collapse supernovae have very massive progenitors, at least 8 times the mass of the Sun, and leave behind neutron stars or black holes. The CO white dwarfs, by contrast, are the remnants of the burnt-out cores of stars that started out with masses below 8 solar masses. These white dwarfs are typically similar in size to Earth but, owing to their high density, their masses are several hundred thousand times larger, between 0.6 and 1.2 times that of the Sun. For a white dwarf to grow to the Chandrasekhar limit, therefore, it must gain matter from somewhere. The most easily conceivable way in which it might do this is by the transfer of mass from a companion star in a binary system.

In the single-degenerate model[6] (Fig. 1), the companion of the white dwarf is a normal, non-degenerate, hydrogen-rich star. If the rate of mass transfer to the white dwarf exceeds 10^{-7} solar masses per year, the deposited layer of hydrogen ignites in steady fusion, and the white dwarf will gradually grow in mass. (At smaller transfer rates, the hydrogen on the white dwarf ignites explosively and the white dwarf is unlikely to grow.) White dwarfs experiencing steady fusion are known as luminous super-soft binary X-ray sources[7], and were first discovered[8] in another galaxy in 1990, and later also in our own Galaxy.

The single-degenerate model has a competitor: the double-degenerate model. In this scheme, two white dwarfs with a combined total mass above the Chandrasekhar limit merge as their orbits shrink through the stars' emission of gravitational waves. For such a system to merge on a timescale shorter than the age of the Universe, it must have been born with an orbital period of less than about 12 hours. In recent years, such close double-white-dwarf systems have been discovered in our Galaxy,

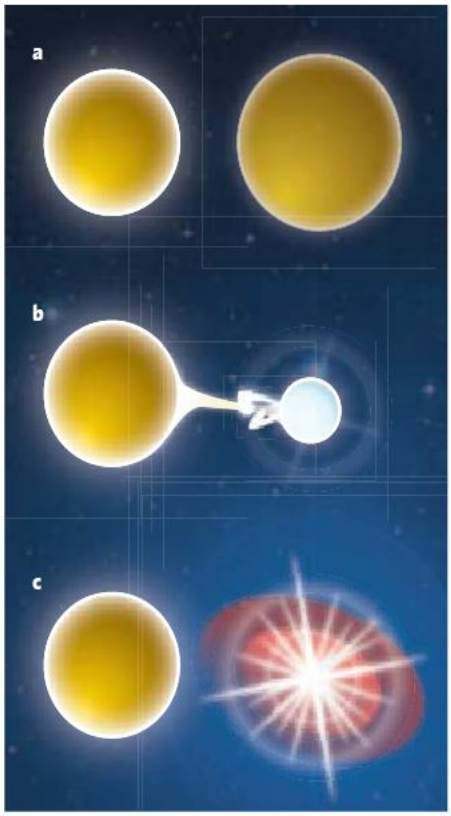

Figure 1 | A degenerate's progress. **a**, In the beginning of the single-degenerate model of how type Ia supernovae arise, two stars of moderate size coexist peacefully in a binary system. The nuclear fuel of the more massive one, which evolves faster, eventually becomes exhausted, causing it to collapse in on itself and become a white dwarf — a core of very dense 'electron-degenerate' matter, mainly in the form of carbon and oxygen. **b**, Through the transfer of mass from the normal star, the mass of the white dwarf increases past the Chandrasekhar limit of 1.39 times the mass of the Sun, and the white dwarf collapses further. **c**, The rapid increase in temperature and density during this collapse triggers the runaway nuclear fusion of carbon and oxygen to (principally) iron, causing the star to blow up in a type Ia supernova explosion, probably leaving no remnant.

although none as yet has the requisite total system mass to exceed the Chandrasekhar limit. Double white dwarfs are so faint that, with current observational capabilities, they cannot be discovered in other galaxies. The luminous super-soft binary systems, by contrast, whose energy output in X-rays alone is typically more than 10,000 times the total energy output of the Sun, are bright enough to be seen at X-ray wavelengths out to distances of several tens of millions of light years.

Voss and Nelemans's discovery[1] of just such a soft X-ray progenitor where a supernova has now been seen is a breakthrough, but raises some puzzling questions. Single-degenerate progenitors are expected to be most prevalent in stellar populations with ages between 0.2 billion and 2 billion years, whereas double-degenerate progenitors should be common in stellar populations of all ages upwards from about 30 million years[9,10]. The stellar population

of the host galaxy where this new discovery was made, NGC 1404, is older than 4 billion years, and so one would have expected many double-degenerate progenitors and very few single-degenerate ones. Thus, given the difficulty of seeing double-degenerate progenitors, one would not expect to have much of a chance of seeing any progenitors in a galaxy of this age.

As the authors discuss[1], the answer might be that there is a hitherto unknown population of younger stars in NGC 1404, or that single-degenerate-type X-ray emission could emerge from lower-mass systems than had been assumed. A further possibility is that the merger of a double-degenerate system with the required mass does not produce a type Ia supernova, but results in the formation of a neutron star[11]. In that case, single-degenerate systems are the sole progenitors of type Ia supernovae, and the fact that they are expected to be comparatively rare in old stellar systems becomes irrelevant. But more highly refined calculations of the physics of double-degenerate mergers are urgently needed before we can draw such a far-reaching conclusion from this single observation. ■

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MOLECULAR BIOLOGY

Cohesin branches out

Frank Uhlmann

The cohesin complex — best known for its role in cell division — does not rest between divisions, and instead participates in regulating gene expression. How it does this is only now becoming clear.

Cohesin is one of the large, ring-shaped protein complexes that constitute a substantial fraction of the chromosomal proteins in nucleated cells. It acts as the chromosomal 'glue', and is thought to encircle and so trap pairs of replicated chromosomes, known as sister chromatids. This allows recognition of these chromatids for segregation during the nuclear-division phase of the cell cycle[1]. But accumulating evidence suggests that cohesin is also involved in regulating gene expression, and that mutations of human cohesin or its regulators cause severe developmental disorders, including Cornelia de Lange syndrome and Roberts syndrome. Six studies[2–7], including one by Wendt *et al.*[4] on page 796 of this issue, provide insight into how cohesin contributes to gene regulation.

The first indication that cohesin does more than pairing sister chromatids came from gene-expression studies[8] in the budding yeast *Saccharomyces cerevisiae*. Cohesin was identified as part of a chromosomal boundary — which consists of molecular elements that separate a region of gene repression from one of active expression. In the fruitfly *Drosophila*, both cohesin and a protein complex that loads cohesin rings onto chromosomes, Nipped-B, have been implicated[9] in gene regulation. Active gene expression often depends on enhancer sequences that are located some distance away from their target genes along the chromosome. In such a setting, cohesin prevents enhancers from activating genes, an effect known as insulation, and Nipped-B alleviates this insulation. Although suggestive, these studies left open the question of whether cohesin directly functions as an insulator, or whether its role in sister-chromatid cohesion has indirect knock-on effects on gene expression.

Two teams[2,3] now show that *Drosophila* neurons — cells that no longer proliferate and so have no sister chromatids in their nuclei — contain cohesin. They also find that inactivation of cohesin specifically in a certain type of neuron in the developing *Drosophila* brain has drastic consequences: expression of the ecdysone hormone receptor is reduced; the neurons fail to eliminate unwanted neuronal projections; and larval development is aborted. These observations clearly indicate that cohesin acts independently of sister-chromatid cohesion. Cohesin is also found in mouse neurons and many other non-dividing cell types[4], opening up the possibility that, in many cases, it contributes to gene regulation.

To determine how cohesin controls gene expression, important questions include where along chromosomes cohesin binds, and what we can learn from its binding pattern. For analysis of such protein–DNA interactions, the technique of chromatin immunoprecipitation (ChIP) is used. The protocol involves chemically crosslinking cohesin to chromosomal DNA, followed by preparing chromatin from the cells, shearing it into small fragments, and purifying cohesin from the mixture. Because of the crosslinking step, the purified cohesin also contains DNA sequences that it had bound to. The DNA-binding sites of cohesin can then be identified by hybridization to DNA microarrays.

First performed[10] in the budding yeast, ChIP analysis revealed that cohesin is loaded onto chromosomes at sites occupied by its loader protein (known as Scc2/4 in *S. cerevisiae*), but afterwards translocates away from the loading sites and accumulates at sites where genes transcribed in opposite directions converge (Fig. 1a). When they reach this final destination, cohesin rings seem to be irreversibly trapped on DNA, as is necessary for stable

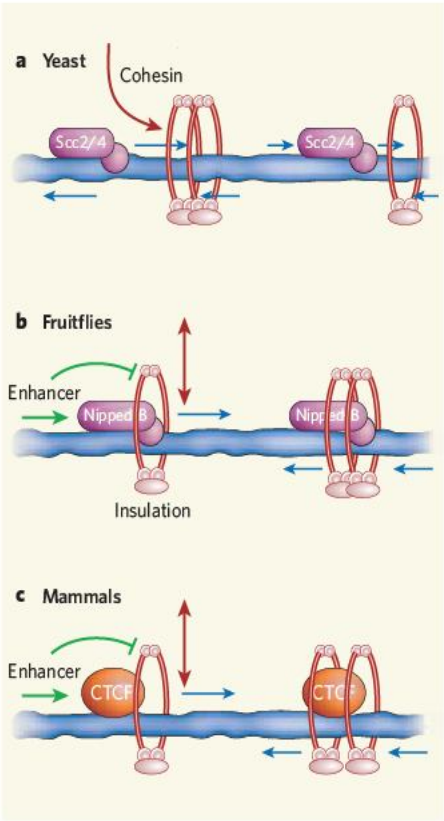

Figure 1 | Cohesin on chromosomes. Depending on the organism, the localization of cohesin complexes along chromosome arms (blue) differs. **a**, In budding yeast, these ring-shaped complexes are helped onto chromosomes by the Scc2/4 loader protein, but translocate away from their loading site, stably anchoring to chromosomal regions where genes transcribed in opposite directions converge (blue arrows). **b**, In the fruitfly, cohesin mostly stays close to its loader Nipped-B, blocking the stimulatory effects of enhancer sequences on gene expression — an effect known as insulation. **c**, Studies[4,5,7] of chromosome binding of mammalian cohesin reveal that these complexes mostly co-localize with the CTCF insulator protein and are required for it to block the activity of enhancer sequences. So mammalian cohesin might act similarly to its fruitfly counterparts.

Figure 2 | Cohesin's role as an insulator. Wendt *et al.*[4] propose that the CTCF insulator protein (orange) might bind to two DNA double strands (red–blue helices) and recruit a cohesin ring. Three subunits of this ring, modelled on known structural features, are indicated in pink, green and light blue. Following its recruitment, the ring may then tether the DNA strands by encircling them. Thus, cohesin complexes could shield genes from the effect of enhancer sequences, regulating gene expression. (Clay model prepared by M. Komata, Tokyo Institute of Technology.)

sister-chromatid cohesion. Similar analyses in *Drosophila* (Fig. 1b) have yielded[6] a seemingly different result, whereby most cohesin remains close to its loader. Thus *Drosophila* cohesin could be remobilized after loading, when insulation of genes from their enhancers is established or eliminated — processes that do not occur to the same extent in the yeast.

The latest ChIP analyses[4,5,7] of mouse and human cohesin reveal another exciting pattern (Fig. 1c). Although the relationship between cohesin and its loader was not investigated in these studies, a striking co-localization between cohesin and a well-known gene regulator, CTCF, is reported. CTCF is a chromosomal-boundary and insulator element, and its close co-localization with cohesin suggests that cohesin might play a part in this protein's function. Indeed, these studies[4,5,7] show that CTCF recruits cohesin, and that, in several instances, cohesin is required for CTCF's insulator function.

How proteins such as CTCF set up chromosome boundaries and insulate enhancers from their genes is still largely mysterious. But now that cohesin has firmly taken the stage, new possibilities emerge. If cohesin rings can embrace two sister chromatids, they might also be able to link a CTCF-binding site to other sequences, or to a second CTCF-binding site, some distance away along the same chromosome. This idea, put forward by Wendt *et al.*[4], is aptly illustrated in Figure 2. Boundary and insulator elements are known to interact with each other, forming clusters in the nucleus, and CTCF contributes to this clustering[11]. Whether cohesin is indeed responsible for tethering CTCF-binding sites can now be tested. Less clear is how clustering of these sites creates domain boundaries and insulators. Nevertheless, cohesin's role as an insulator could explain its contribution to gene regulation and human developmental disorders.

The next question, then, is how its ability to tether CTCF sites relates to cohesin's role in sister-chromatid cohesion. Most human cohesin complexes dynamically associate with chromosomes[12], which could aid the establishment and disassembly of gene-regulatory interactions. After sister-chromatid formation, a part of cohesin becomes stably trapped on chromosomes, probably to provide enduring cohesion between sister chromatids. Are the same sites used for both gene regulation and sister-chromatid cohesion? And would stable binding at such sites be compatible with the possible need for changes in the gene-expression programme? Did the genome-wide analyses[4–6] detect all sites of cohesin association, or could more cohesin be spread out over large intergenic regions? Although we have gained exciting insight into how architectural components of chromosomes contribute to gene regulation, further fascinating questions remain to be addressed. ■

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CATALYSIS

The art of splitting water

Thomas J. Meyer

Plants produce oxygen from water, but the same chemical reaction is hard to achieve synthetically. A new family of catalysts could breathe fresh life into the quest for artificial photosynthesis.

Photosynthesis in plants underpins the existence of many life-forms on Earth. At its heart is a remarkable chemical reaction: the light-powered conversion of water and carbon dioxide into oxygen and carbohydrates. The development of an artificial version of this reaction, based on splitting water into oxygen and hydrogen, is highly desirable, not least because of hydrogen's attraction as a fuel. Reporting in the *Journal of the American Chemical Society*, Bernhard and colleagues[1] describe the preparation of a new family of synthetic catalysts for the first part of this splitting reaction — water oxidation. The reactivity of the iridium-based catalysts that they have developed can be modified simply by varying the organic framework surrounding the metal.

Given the breakthroughs reported seemingly daily in materials science, nanotechnology and molecular biology, it might seem odd that we have made so little progress in designing catalysts for essential reactions such as water oxidation under conditions comparable to those of photosynthesis. The problem is that water oxidation is inherently difficult. It demands the loss of four electrons and four protons from two water molecules, all accompanied by the formation of an oxygen–oxygen bond (Fig. 1a). Removing one electron at a time doesn't work because it produces a high-energy hydroxyl radical and is much too slow (Fig. 1b).

Thankfully, water oxidation occurs all around us during photosynthesis in green plants and certain bacteria — at least when the sun is shining. In photosynthesis, light is absorbed by antenna arrays found in photosystem II, part of the photosynthetic apparatus within the chloroplasts of green leaves. The light-excited antenna transfers its energy to a chlorophyll molecule in a neighbouring protein complex, known as the reaction centre. The excitation of the chlorophyll is quenched by transferring electrons to acceptor molecules

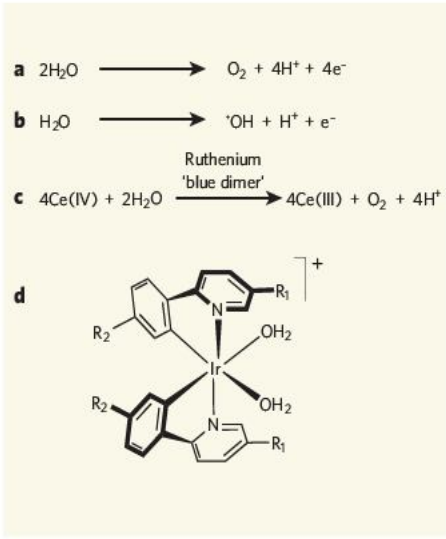

Figure 1 | Catalytic water oxidation. Photosynthesis is fuelled by the conversion of water into oxygen and hydrogen. **a**, In the first part of this process, known as water oxidation, water is converted into oxygen, four protons (H^+) and four electrons (e^-). **b**, Oxidation by the removal of one electron at a time is slow because it creates an energetically unfavourable intermediate, a hydroxyl radical ($^•OH$). **c**, One of the few artificial catalysts for water oxidation is the ruthenium 'blue dimer', which is activated by a cerium salt (shown here as Ce(IV), reduced in the reaction to a lower oxidation state, Ce(III)). **d**, Bernhard and colleagues[1] describe a new family of iridium (Ir) catalysts for water oxidation, shown here. Their activity can be tuned by changing the organic molecules bound to the metal. R_1 and R_2 represent general chemical groups.

elsewhere in the photosystem. Electron-deficient molecules (oxidants) are thus created that can accept electrons from neighbours.

The source of these electrons is a part of the reaction centre known as the oxygen-evolving complex, or OEC. The OEC makes up its lost electrons by stealing some from water molecules, producing oxygen in the process[2–4]. Even plants find this task difficult: under ambient sunlight in the chloroplast, the OEC must be resynthesized every half an hour or so owing to the oxidation damage it suffers from the oxygen that it has produced.

A synthetic, non-protein catalyst that can oxidize water as effectively as the OEC does exist. This is the ruthenium 'blue dimer'[5,6], a complex that must be activated electrochemically or by a strong oxidizing agent (usually a cerium salt; Fig. 1c). The blue dimer is a well-defined molecule that undergoes the stepwise loss of four electrons and four protons, producing a fleeting intermediate that rapidly oxidizes water. But although initially effective, the blue dimer can lose its catalytic efficiency after just a few cycles.

Compared with earlier catalysts such as the blue dimer, Bernhard and colleagues' iridium-based catalysts[1] (Fig. 1d) are simple to make. Their oxidizing ability is tuned by changing the ligand molecules that bind to the iridium metal. As with the blue dimer, they need to be activated by a cerium oxidizing agent. The details of this mechanism are still lacking, but proton-coupled electron transfer is likely to be essential. This trick of the trade is used by both the OEC and the ruthenium blue dimer, and involves simultaneous transfers of both electrons and protons as the catalyst is activated. This allows many oxidative equivalents to accumulate at one site, one step at a time, without a catastrophic build-up of charge[7].

Barring serendipitous discoveries, further progress in designing catalysts for water oxidation will require detailed knowledge of the mechanism by which these reactions occur. Bernard and colleagues' iridium compounds[1] are promising. But we have limited understanding of how they work, and their catalysed reactions are slow — more than 100 million times slower than those in the OEC. Nevertheless, catalysts for water oxidation are so rare that the discovery of a new family is cause for celebration. Once the essential design details that govern their behaviour have been thrashed out, and their stability is improved, they offer real prospects as tunable catalysts for the essential process of water oxidation. ■

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EVOLUTIONARY BIOLOGY

Darwin in the fossils

Andrew P. Hendry

Adaptation by natural selection is thought to drive evolution. Although it has been difficult to confirm this process in the fossil record, evidence has been there all along: we just haven't been looking properly.

Most biologists accept that morphological evolution reflects the darwinian process of natural selection, with evidence coming from numerous studies of contemporary populations[1] and from classic interpretations of the fossil record[2]. Some palaeontologists, however, see a fly in this darwinian ointment. In particular, statistical analyses of fossil data generally fail to confirm that natural selection strongly influences morphological evolution[3–5]. Partly for this reason, a cadre of scientists is convinced that natural selection is less prevalent and important than typically assumed. The latest work from Gene Hunt and colleagues[6,7] may lessen this dissent.

A standard approach to analysing fossil sequences is to infer evolutionary mechanisms from temporal patterns in phenotypic traits — average body size, for example. One such pattern is a reasonably consistent directional trend. For example, body size might increase through time because larger body size is favoured most of the time ('directional selection'). Another pattern is constancy, or 'stasis'. For example, body size might remain the same through time because the optimal — that is, best adapted — body size does not change much through time ('stabilizing selection'). The third pattern is randomness. For example, body size might change unpredictably through time because the optimal body size is changing unpredictably ('variable selection'), or because there is no optimum at all and so body size drifts according to the arbitrary success of different individuals.

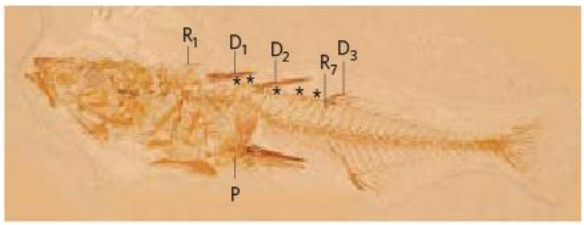
Although natural selection can cause all three patterns (directional trends, stasis or randomness), the tradition has been to invoke selection only when models of randomness fit the data very poorly. Up to now, few studies have been able to reject randomness; and those that have point to stabilizing selection, rather than directional selection. Taken at face value, these results might suggest that organisms have evolved their distinctive phenotypes without much aid from directional selection. If so, darwinian mechanisms might not be particularly important in generating the diversity of life.

The question to be addressed is whether directional selection really is absent in the fossil record, or whether the standard methods of analysis are simply biased against its detection. To address this question, one might first apply the standard battery of methods to an exemplary fossil sequence where directional selection is almost certain to be important. A good candidate is Mike Bell's 21,500-year record[4] of defensive armament in fossil sticklebacks from an ancient Nevada lake (Box 1, overleaf). This data set includes more than 5,000 stickleback specimens grouped into 250-year intervals, a remarkable level of resolution and replication. At one point in the time sequence, heavily armoured sticklebacks colonized the lake and

BOX 1 | Defensive armament of threespine sticklebacks

Threespine sticklebacks (*Gasterosteus* spp.) are most notable to evolutionary biologists for their diverse adaptations to specific freshwater environments[8]. Remarkably, most of this adaptive radiation took place after the last glaciation, a period of about 10,000 years, or 5,000 stickleback generations.

Of particular interest here, sticklebacks at sites with predatory fishes retain defensive armament, including spines along the back and on the pelvis. In contrast, sticklebacks at sites with few predatory fishes evolve much reduced armament.



Hunt *et al.*[7] studied fossils of a heavily armoured population of *Gasterosteus doryssus* that colonized a lake with few predatory fishes. The traits studied were the number of dorsal spines (D in the figure here), the size and structure of the pelvis (P), and the number of overlapping bones that help support the dorsal spines ('touching pterygiophores', asterisks between R_1 and R_7). Each of these traits is expected to decrease when an armoured population colonizes a site with few predatory fishes. **A.H.**

then showed a steady reduction in armament (Fig. 1). This evolutionary change almost certainly reflects natural selection because, in addition to other reasons, predatory fishes are rare in this ancient lake, and modern sticklebacks evolve reduced armour in the absence of fish predation[8].

If the standard methods were ever to diagnose directional selection in the fossil record, then surely it would be for these little fishes from Nevada. But not so — Bell and colleagues[4] found that the existing methods inferred randomness almost every time. It is often said that when a pattern is not visible without statistics, then that pattern isn't worth discussing. But here we have a pattern that is logical and manifestly obvious (Fig. 1) but cannot be confirmed by statistics. With more than a hint of resignation, Bell *et al.*[4] concluded that "current methods to study rates or patterns of phenotypic evolution in the fossil record are strongly biased against detecting directional selection". Taking up this challenge, Hunt[5–7] refined the existing methods and, with Bell and Mike Travis[7], used these methods to provide strong support for directional selection in the stickleback fossils.

One of Hunt's refinements was to overturn the usual burden of proof, wherein randomness has been assumed by default and retained as the evolutionary inference unless overwhelmingly rejected in statistical tests. But there is no biological reason for this *a priori* ascendancy of randomness, and randomness is extremely difficult to reject with the existing methods[3]. Instead, we should be comparing, on equal footing, the fit of different evolutionary models (such as directional selection, stabilizing selection or randomness) to the observed data[5–7]. That is, we should stop striving to reject the null hypothesis of randomness, and instead weigh the level of statistical support for alternative models.

Hunt's other refinement was to test a more realistic model of selection. Previous tests have tended to treat directional selection as a reasonably consistent force driving average phenotypes in a given direction. This model is obviously unrealistic in the absence of any force expected to sustain selection in a particular direction over such long time frames. Instead, adaptation should often involve the asymptotic approach of phenotypes towards a particular optimum, near which the average should then remain until the optimum is perturbed[1,9,10]. That is, environmental change should cause initially strong directional selection that should gradually grade into stabilizing selection, a 'hybrid' selection model if you will. This particular process is what would be expected for heavily armoured sticklebacks colonizing a lake where predatory fishes are rare, and the hybrid model provided an excellent fit to the fossil stickleback data (Fig. 1).

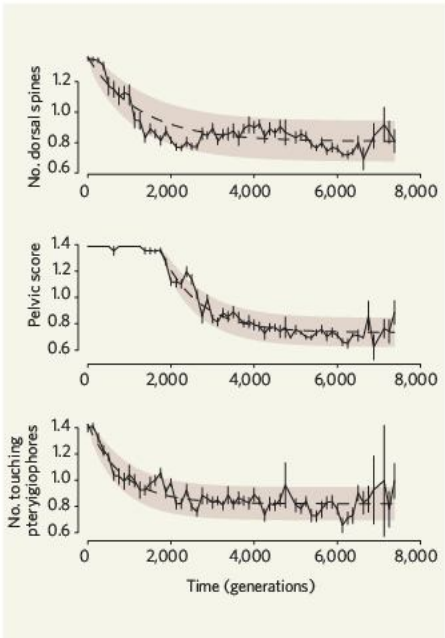


Figure 1 | Evolutionary change in the defensive armament of fossil sticklebacks. The features concerned (the number of dorsal spines, the size and structure of the pelvis and the number of 'touching pterygiophores') are indicated in Box 1. The *x*-axis shows the number of stickleback generations; data points are the average trait size for each 250-year time interval; vertical bars are standard errors. The dotted line is the best-fit relationship for the hybrid model of selection tested by Hunt *et al.*[7], and the grey envelope shows the 95% confidence interval for the model fit. (Figure modified from ref. 7.)

Several potential criticisms need to be addressed. First, Hunt *et al.*[7] start their analysis at exactly the point in time when each armour trait begins to decrease, which favours a model of initially strong directional selection. But this choice does not undermine their general conclusion, because the standard methods could not reject randomness even when started at these same times[4]. Second, the analysis[7] of the stickleback data formally examined only one model of selection — the hybrid directional–stabilizing model they expected beforehand. The authors are here again stacking the deck for success in confirming selection. But then this is the point. Their analysis is akin to a positive control in showing that a new statistical method can infer the correct evolutionary process when that process is almost certain to be acting.

The obvious next step is to apply similar thinking[5–7] to a much larger array of fossil data and evolutionary models. Doing so will justifiably accelerate the retreat from a 'one model to rule them all' vision. This work will almost certainly generate additional support from fossil sequences for the action of natural selection. Perhaps more importantly, it will become easier for biologists to accept randomness when random models still receive the most support. This acceptance, however, needs to be tempered by the realization that selection can certainly generate patterns that look random. Particularly valuable for all this work will be more fossil data with fine temporal resolution such as that seen in the stickleback samples, because selection can cause noteworthy changes in less than a hundred years[11]. Ultimately, we might hope for the emergence of general conclusions about the role of natural selection in generating the diversity of life. ■

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OBITUARY

M. Judah Folkman (1933–2008)

Scientist, surgeon and creator of the field of angiogenesis research.


K. JOHNSON

Judah Folkman often said "Science goes where you imagine it." Few imagined more boldly or pushed science further than he did. As director of the Vascular Biology Program and a former surgeon-in-chief at the Children's Hospital Boston in Massachusetts, Folkman brought both a scientist's and a surgeon's perspectives to finding solutions to medical problems. His research observation that some tumours grow whereas others remain dormant, fused with his clinical experience in removing hot, bloody malignancies, produced a profound insight — that the recruitment of a dedicated blood supply, a process known as angiogenesis, is essential to tumour growth.

Folkman's single-mindedness in demonstrating this principle redefined our understanding of cancer biology. Furthermore, it established angiogenesis as a fundamental biological process operating in health, embryonic development and many diseases besides cancer — macular degeneration, heart disease, diabetic retinopathy, endometriosis and obesity among them. Peter Carmeliet has predicted that treatments to stimulate or repress angiogenesis will eventually benefit half a billion people worldwide (P. Carmeliet *Nature* **438**, 932–936; 2005). Today, thousands of researchers around the world, many trained in Folkman's lab, are working to make this promise a reality. But when Folkman embarked on the scientific odyssey that would establish the field of angiogenesis, he sailed alone.

Folkman was born in 1933, in Cleveland, Ohio, and was educated at Ohio State University and Harvard Medical School. He followed this with a surgical residency at Massachusetts General Hospital. His angiogenesis hypothesis had its roots in the late 1960s. On leave from his surgical residency to complete two years of military service at the National Naval Medical Center in Bethesda, Maryland, he was developing blood substitutes and testing them by perfusing rabbit thyroid glands in the lab. Out of curiosity, he seeded the glands with mouse tumour cells. The tumours grew to about 1 millimetre, then stopped. Yet, *in vivo*, the same cells formed large, lethal cancers.

After completing his surgical training and establishing his lab at the Children's Hospital, Folkman pursued the puzzle of dormant versus active tumours. Watching thread-like capillaries grow straight towards dormant tumours, he concluded that the tumours secreted an angiogenesis stimulator. Because not all tumours become vascularized, however, he further proposed a corollary process of angiogenesis inhibition. Blocking angiogenesis, he argued, would provide a new approach to controlling certain cancers.

Folkman published his ideas about angiogenesis in 1971 (J. Folkman *N. Engl. J. Med.* **285**, 1182–1186; 1971). This landmark paper initially met with scepticism. Undaunted, he and his small research group developed the cell-culture methods, bioassays and drug-delivery systems they needed to validate the theory. Notably, the technology for slow-release, drug-delivery polymers emerged from this work.

It took a decade for Folkman's lab to isolate the first angiogenesis stimulator — basic fibroblast growth factor (bFGF) — and yet another for it to identify the inhibitors angiostatin and endostatin. These breakthroughs launched an era of discovery and validation, during which Folkman's group and others uncovered additional pro- and antiangiogenic factors, began mapping the molecular pathways of pathological angiogenesis, and started to develop antiangiogenic drugs. Today, more than 50 angiogenesis inhibitors are approved or in clinical trials around the globe. Although most target cancer, drugs to treat a condition called wet age-related macular degeneration have had the most stunning success so far, reversing blindness in many patients.

Folkman never stopped pushing his own imagination or the bounds of scientific understanding. In 2004, he proposed that we may someday control cancer by "treating a biomarker" with angiogenesis inhibitors (J. Folkman & R. Kalluri *Nature* **427**, 787; 2004), just as we treat heart disease by prescribing statins in response to high cholesterol levels. Folkman foresaw biomarkers for the 'angiogenic switch', the point at which the balance of angiogenesis stimulators and inhibitors shifts in favour of stimulation, generating the web of blood vessels that turns cancer into a killer. The switch happens years before a tumour can be imaged or felt. Folkman speculated that if cancer were detected this early and treated with low-toxicity antiangiogenic drugs, it would remain dormant. Patients would have cancer but not disease.

At the time of his sudden death on 14 January, Folkman had been testing this theory in patients at risk of recurrent cancer, using biomarkers for early angiogenesis developed in his Vascular Biology Program. He had also begun to view angiogenesis as an organizing principle of biology, essential for the growth of any mass from a cancerous tumour to an atherosclerotic plaque. He had proposed that angiogenesis biomarkers could potentially detect a range of blood-vessel-dependent diseases, and that one day a single, broad-spectrum angiogenesis inhibitor or a combination of antiangiogenic drugs might be used to treat them all. This was another bold theory, one left for others to pursue.

Folkman's contributions to vision and cardiovascular research, as well as to cancer biology, were widely recognized. Apart from being elected to the National Academy of Sciences and Institute of Medicine, and appointed to the President's Cancer Advisory Board, he received more than 150 awards and prizes, including scientific honours from 11 nations. But neither the awards nor accounts of his scientific accomplishments capture what those who knew him valued most in Folkman — his humanity and generosity. He was legendary for sharing unpublished data, and masterly at balancing guidance and creative freedom to nurture young investigators' careers.

Nowhere was Folkman's compassion and generosity more evident, however, than with patients. He would leave the office late in the evening, a briefcase slung over each shoulder, a notebook of to-do's in his breast pocket, wheeling his laptop behind him. After dinner with his wife of 47 years, Paula, he would retire to his study, take out the notebook, and begin calling patients who had left messages that day. Most were people he had never met, desperate for hope and advice. He called them all.

Folkman never used his first name, Moses, but he shared much in common with his Biblical namesake. He was a teacher, leader and iconoclast. Although he did not live to see the full promise of antiangiogenic therapy realized, he trained and inspired many who will carry forward his dream.

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ARTICLES

The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans

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Choanoflagellates are the closest known relatives of metazoans. To discover potential molecular mechanisms underlying the evolution of metazoan multicellularity, we sequenced and analysed the genome of the unicellular choanoflagellate *Monosiga brevicollis*. The genome contains approximately 9,200 intron-rich genes, including a number that encode cell adhesion and signalling protein domains that are otherwise restricted to metazoans. Here we show that the physical linkages among protein domains often differ between *M. brevicollis* and metazoans, suggesting that abundant domain shuffling followed the separation of the choanoflagellate and metazoan lineages. The completion of the *M. brevicollis* genome allows us to reconstruct with increasing resolution the genomic changes that accompanied the origin of metazoans.

Choanoflagellates have long fascinated evolutionary biologists for their marked similarity to the 'feeding cells' (choanocytes) of sponges and the possibility that they might represent the closest living relatives of metazoans[1,2]. Over the past decade or so, evidence supporting this relationship has accumulated from phylogenetic analyses of nuclear and mitochondrial genes[3–6], comparative genomics between the mitochondrial genomes of choanoflagellates, sponges and other metazoans[7,8], and the finding that choanoflagellates express homologues of metazoan signalling and adhesion genes[9–12]. Furthermore, species-rich phylogenetic analyses demonstrate that choanoflagellates are not derived from metazoans, but instead represent a distinct lineage that evolved before the origin and diversification of metazoans (Fig. 1a, Supplementary Fig. 1 and Supplementary Note 3.1)[8,13]. By virtue of their position on the tree of life, studies of choanoflagellates provide an unparallelled window into the nature of the unicellular and colonial progenitors of metazoans[14].

Choanoflagellates are abundant and globally distributed microbial eukaryotes found in marine and freshwater environments[15,16]. Like sponge choanocytes, each cell bears an apical flagellum surrounded by a distinctive collar of actin-filled microvilli, with which choanoflagellates trap bacteria and detritus (Fig. 1b). Using this highly effective means of prey capture, choanoflagellates link bacteria to higher trophic levels and thus have critical roles in oceanic carbon cycling and in the microbial food web[17,18].

More than 125 choanoflagellate species have been identified, and all species have a unicellular life-history stage. Some can also form simple colonies of equipotent cells, although these differ substantially from the obligate associations of differentiated cells in metazoans[19]. Studies of basal metazoans indicate that the ancestral metazoan was multicellular and had differentiated cell types, an epithelium, a body plan and regulated development including gastrulation. In contrast, the last common ancestor of choanoflagellates and metazoans was unicellular or possibly capable of forming simple colonies, underscoring the abundant biological innovation that accompanied metazoan origins.

Despite their evolutionary and ecological importance, little is known about the genetics and cell biology of choanoflagellates. To gain insight into the biology of choanoflagellates and to reconstruct the genomic changes attendant on the early evolution of metazoans, we sequenced the genome of the choanoflagellate *M. brevicollis* and compared it with genomes from metazoans and other eukaryotes.

Gene structure and intron evolution

The ~41.6 megabase (Mb) *M. brevicollis* genome contains approximately 9,200 genes (Supplementary Notes 1 and 2) and is comparable in size to the genomes of filamentous fungi (~30–40 Mb) and other free-living unicellular eukaryotes (for example, small diatoms at ~20–35 Mb[20] and ichthyosporeans at ~20–25 Mb[21]). Metazoan genomes are typically larger, with few exceptions[22].

M. brevicollis genes have several distinguishing structural features (Table 1). Whereas the *M. brevicollis* genome is compact, its genes are almost as intron-rich as human genes (6.6 introns per *M. brevicollis* gene versus 7.7 introns per human gene). *M. brevicollis* introns are short (averaging 174 bp) relative to metazoan introns, and with few exceptions do not include the extremely long introns found in some metazoan genes (Supplementary Fig. 2 and Supplementary Note 3.3).

Comparisons of intron positions in a set of conserved genes from *M. brevicollis*, diverse metazoans and representative intron-rich fungi, plants and a ciliate reveal that the last common ancestor of

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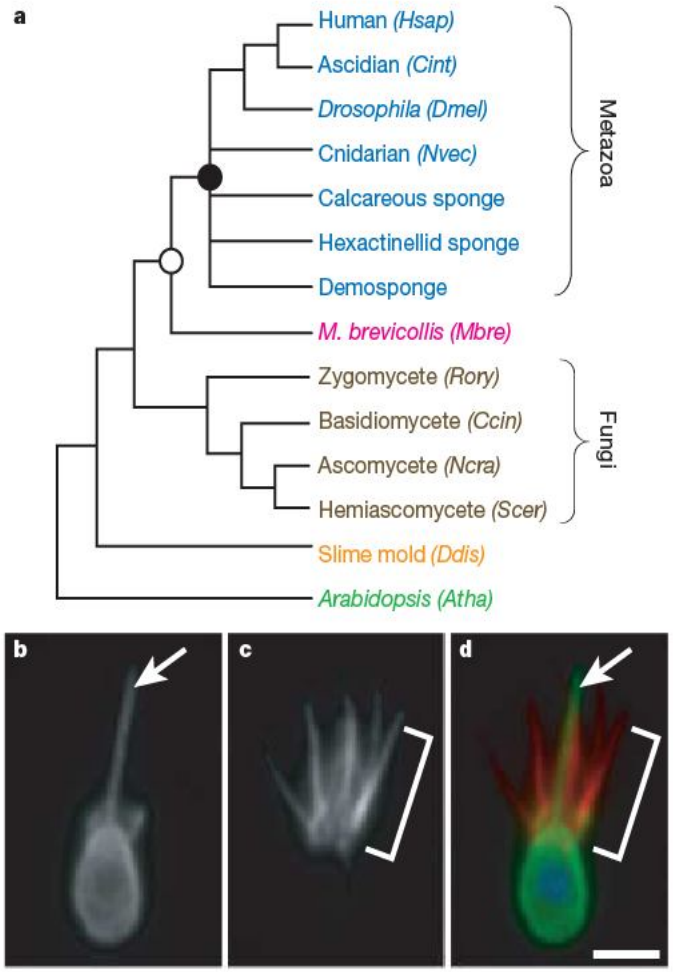

Figure 1 | Introduction to the choanoflagellate *Monosiga brevicollis*. **a**, The close phylogenetic affinity between choanoflagellates and metazoans highlights the value of the *M. brevicollis* genome for investigations into metazoan origins, the biology of the last common ancestor of metazoans (filled circle) and the biology of the last common ancestor of choanoflagellates and metazoans (open circle). Genomes from species shown with their abbreviation were used for protein domain comparisons in this study: human (*Homo sapiens*, *Hsap*), ascidian (*Ciona intestinalis*, *Cint*), *Drosophila* (*Drosophila melanogaster*, *Dmel*), cnidarian (*N. vectensis*, *Nvec*), *M. brevicollis* (*Mbre*), zygomycete (*Rhizopus oryzae*, *Rory*), basidiomycete (*Coprinus cinereus*, *Ccin*), ascomycete (*Neurospora crassa*, *Ncra*), hemiascomycete (*Saccharomyces cerevisiae*, *Scer*), slime mould (*Dictyostelium discoideum*, *Ddis*) and *Arabidopsis* (*Arabidopsis thaliana*, *Atha*). **b–d**, Choanoflagellate cells bear a single apical flagellum (arrow, **b**) and an apical collar of actin-filled microvilli (bracket, **c**). **d**, An overlay of β-tubulin (green), polymerized actin (red) and DNA localization (blue) reveals the position of the flagellum within the collar of microvilli. Scale bar, 2 μm.

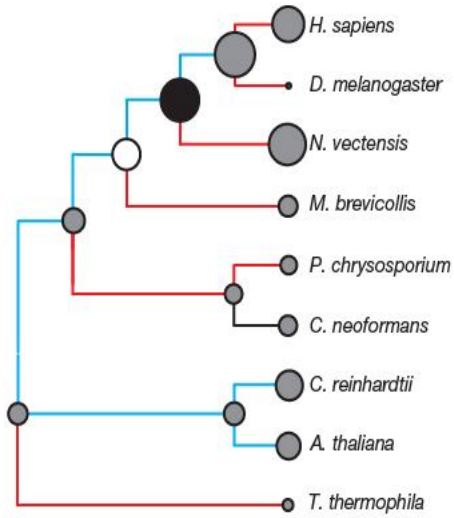

Figure 2 | Intron gain preceded the origin and diversification of metazoans. Ancestral intron content, intron gains and intron losses were inferred by the Csuros maximum likelihood method[45] from a sample of 1,054 intron positions in 473 highly conserved genes in representative metazoans (humans, *D. melanogaster* and *N. vectensis*), *M. brevicollis*, intron-rich fungi (*Cryptococcus neoformans A* and *Phanerochaete chrysosporium*), plants and green algae (*A. thaliana* and *Chlamydomonas reinhardtii*), and a ciliate (*Tetrahymena thermophila*). Branches with more gain than loss are blue, those with more loss than gain are red, and those with comparable amounts of each are black. The inferred or observed number of introns present in ancestral and extant species is indicated by proportionally sized circles. As in Fig. 1, the last common ancestor of metazoans and the last common ancestor of choanoflagellates and metazoans are represented by a filled circle and an open circle, respectively. Other ancestral nodes are indicated by grey circles.

choanoflagellates and metazoans had genes at least as intron-rich as those of modern choanoflagellates (Fig. 2, Supplementary Figs 3 and 4, and Supplementary Note 3.3). Notably, these analyses reveal that the eumetazoan ancestor contained a substantially higher density of introns than the last common ancestor of choanoflagellates and metazoans. This is consistent with a proliferation of introns during the early evolution of the Metazoa[23].

Premetazoan history of protein domains required for multicellularity

The *M. brevicollis* genome provides unprecedented insight into the early evolution of metazoan genes. Annotation of the *M. brevicollis* genome using Pfam and SMART (two protein domain prediction databases) reveals 78 protein domains that are exclusive to choanoflagellates and metazoans, only two of which have been reported previously in choanoflagellates (Supplementary Table 4)[10]. Because genomic features shared by *M. brevicollis* and metazoans were probably present in their last common ancestor, this study extends the evolutionary history of a cohort of important protein domains to the premetazoan era. Many of these domains are central to cell signalling and adhesion processes in metazoans, suggesting a role in the origin of multicellularity. In contrast, metazoan genomic features that are missing from the *M. brevicollis* genome may have evolved within the metazoan lineage, or may have existed in the last common ancestor with choanoflagellates and were subsequently lost on the stem leading to *M. brevicollis*. Presumably, there are many genomic features that evolved in the metazoan lineage, and the *M. brevicollis* genome provides our first glimpse at the complement of genes and protein domains that predate metazoan origins.

To investigate further the extent to which molecular components required for metazoan multicellularity evolved before the origin of

Table 1 | *M. brevicollis* genome properties in a phylogenetic context

	Metazoa				Choanoflagellates	Fungi		*Dictyostelium*	Plants
	Hsap	*Cint*	*Dmel*	*Nvec*	*Mbre*	*Ccin*	*Ncra*	*Ddis*	*Atha*
Genome size (Mb)	2,900	160	180	357	42	38	39	34	125
Total number of genes	23,224	14,182	14,601	18,000	9,196	13,544	9,826	13,607	27,273
Mean gene size (bp)	27,000	4,585	5,247	6,264	3,004	1,679	1,528	1,756	2,287
Mean intron density (introns per gene)	7.7	6.8	4.9	5.8	6.6	4.4	1.8	1.9	4.4
Mean intron length (bp)	3,365	477	1,192	903	174	75	136	146	164
Gene density (kb per gene)	127.9	11.9	13.2	19.8	4.5	2.7	4.0	2.5	4.5

Species names follow the four-letter convention from Fig. 1.

metazoans, we performed targeted searches in the *M. brevicollis* genome and representative metazoan, fungal and plant genomes for homologues of critical metazoan cell adhesion, cell signalling and transcription factor protein families.

An abundance of cell adhesion domains

A critical step in the transition to multicellularity was the evolution of mechanisms for stable cell adhesion. *M. brevicollis* encodes a diverse array of cell adhesion and extracellular matrix (ECM) protein domains previously thought to be restricted to metazoans (Fig. 3). At least 23 *M. brevicollis* genes encode one or more cadherin domains, homologues of which are required for cell sorting and adhesion during metazoan embryogenesis[24], and 12 genes encode C-type lectins, 2 of which are transmembrane proteins. Whereas soluble C-type lectins have functions ranging from pathogen recognition to ECM organization, transmembrane C-type lectins mediate specific adhesive activities such as contact between leukocytes and vascular endothelial cells, cell recognition, and molecular uptake by endocytosis[25–27].

The genome of *M. brevicollis* also contains integrin-α and immunoglobulin domains—cell adhesion domains formerly thought to be restricted to metazoans. In metazoans, integrin-α- and integrin-β-domain-containing proteins heterodimerize before binding to ECM proteins such as collagen[28]. We find that *M. brevicollis* has at least 17 integrin-α-domain-containing proteins, but no integrin-β domains. Metazoan immunoglobulin-domain-containing proteins have both adhesive and immune functions. The *M. brevicollis* genome encodes a total of five immunoglobulin domains that show affinity for the I-set, V-set or C2-set subfamilies, but not the vertebrate-specific C1-set subfamily. In contrast to *M. brevicollis*, metazoan genomes possess from ~150 to ~1,500 immunoglobulin domains (Supplementary Table 7), suggesting that the radiation of the immunoglobulin superfamily occurred after the divergence of choanoflagellates and metazoans.

The finding in *M. brevicollis* of cell adhesion domains that were previously known only in metazoans has two important implications. First, the common ancestor of metazoans and choanoflagellates possessed several of the critical structural components used for multicellularity in modern metazoans. Second, given the absence of evidence for stable cell adhesion in *M. brevicollis*, this also suggests that homologues of metazoan cell adhesion domains may act to mediate interactions between *M. brevicollis* and its extracellular environment.

Extracellular-matrix-associated protein domains

As the targets of many adhesion receptors, the question of whether metazoan-type ECM proteins and domains evolved before or after the transition to multicellularity is of great interest. In metazoans, collagens are ECM proteins that polymerize to form a major component of the basement membrane of epithelia and have been invoked as a potential 'key innovation' during the transition to multicellularity[29]. We find five collagen-domain-encoding genes in the *M. brevicollis* genome, two of which encode the diagnostic Gly-X-Y repetitive sequence motif (where X and Y are frequently proline and hydroxyproline, respectively) in an arrangement similar to metazoan collagens[30]. Other ECM-associated domains previously known only from metazoans that occur in *M. brevicollis* include laminin domains (an important class that contributes to the basement membrane), the reeler domain (found in the neuronal ECM protein reelin[31]) and the ependymin domain (an extracellular glycoprotein found in cerebrospinal fluid[32]; Fig. 3 and Supplementary Table 4).

The discovery of putatively secreted ECM proteins in a free-living choanoflagellate suggests that elements of the metazoan ECM evolved in contact with the external environment before being sequestered within an epithelium. Although some choanoflagellates secrete extracellular structures or adhere to form colonial assemblages[19,33,34], *M. brevicollis* is not known to do so. Instead, these ECM protein homologues in *M. brevicollis* may mediate an analogous process such as substrate attachment.

Against the backdrop of abundant conservation of cell adhesion and ECM protein domains among the genomes of *M. brevicollis* and metazoans, it is important to note the differences. Individual cell adhesion and ECM-associated domains in the *M. brevicollis* genome often occur in unique arrangements, and clear orthologues of specific metazoan adhesion proteins are rarely found. Although the domains associated with metazoan adhesion and ECM proteins were present in the ancestor of choanoflagellates and metazoans, the canonical metazoan adhesion protein architectures[35] probably evolved after the divergence of the two lineages.

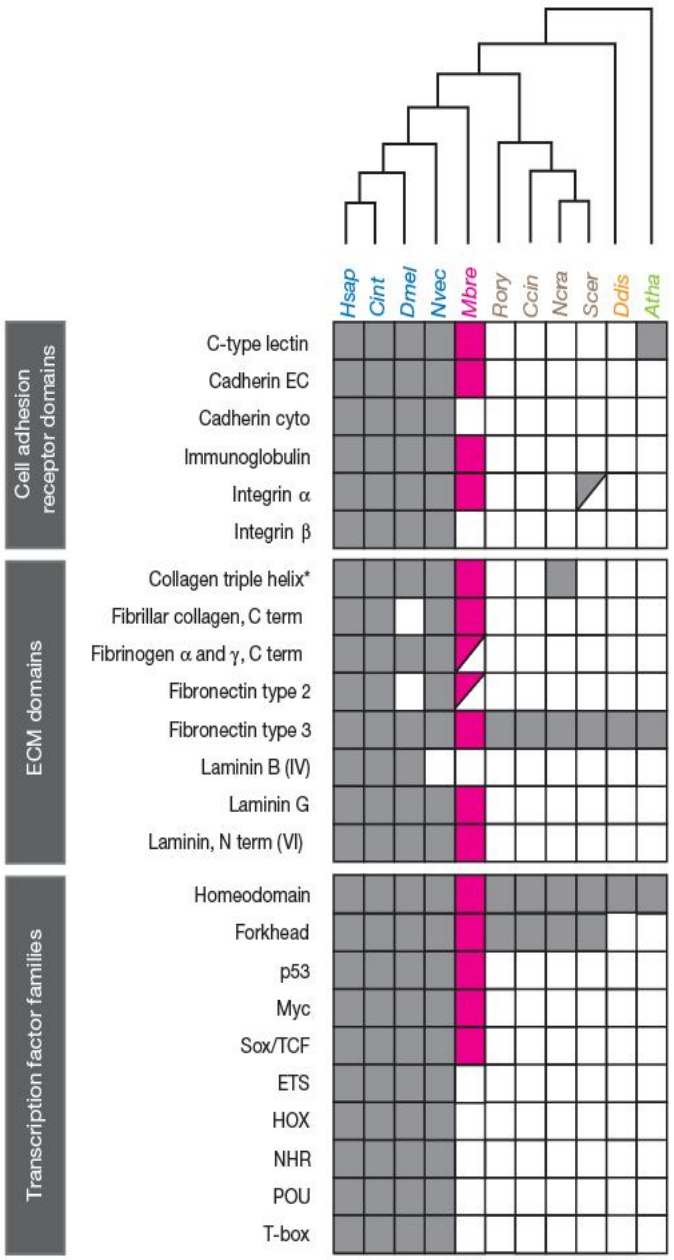

Figure 3 | Phylogenetic distribution of metazoan-type cell adhesion domains and sequence-specific transcription factor families. *M. brevicollis* possesses diverse adhesion and ECM domains previously thought to be unique to metazoans (magenta). In contrast, many metazoan sequence-specific transcription factors are absent from the *M. brevicollis* gene catalogue. For adhesion and ECM domains, a filled box indicates a domain identified by both SMART and Pfam[37,48], a half-filled box indicates a domain identified by either SMART or Pfam, and an open box indicates a domain that is not encoded by the current set of gene models. The presence (filled box) or absence (empty box) of transcription factor families was determined by reciprocal BLAST and SMART/Pfam domain annotations (Supplementary Note 3.5). Species names follow the convention from Fig. 1. EC, extracellular domain; cyto, cytoplasmic domain; asterisk, collagen triple-helix-domains occur in the extended tandem arrays diagnostic of collagen proteins found only in metazoans and choanoflagellates.

Domain shuffling in the evolution of intercellular signalling pathways

Our analysis of the *M. brevicollis* genome reveals little evidence that metazoan-specific signalling pathways were present in the last common ancestor of choanoflagellates and metazoans. Many pathways are missing entirely, and *M. brevicollis* genes with some similarity to metazoan signalling machinery are largely found to share conserved domains without aligning across the full span of what are often complex multidomain proteins (for example, epidermal growth factor (EGF) repeats are common to Notch, and also to many other proteins; Supplementary Table 8). Specifically, no receptors or ligands were identified from the NHR (nuclear hormone receptor), WNT and TGF-β signalling pathways. The only evidence of the JAK (Janus kinase)/STAT (signal transducer and activator of transcription) pathway is an apparent *STAT*-like gene that encodes a STAT DNA-binding domain and a partial SH2 domain. Convincing evidence is also lacking for the Toll signalling pathway—a signalling system important both for development and for innate immunity in metazoans.

Nonetheless, the genome of *M. brevicollis* does provide insights into the evolution of Notch and hedgehog (Hh) signalling pathways. Cassettes of protein domains found in metazoan Notch receptors (EGF, NL and ANK (ankyrin repeats)) are encoded on separate *M. brevicollis* genes in arrangements that differ from metazoan Notch proteins, and definitive domains, such as the NOD domain and the MNNL region, are absent (Fig. 4a).

Homologues of hedgehog, dispatched and patched genes are also present; however, there is no evidence for smoothened nor for its defining frizzled domain. In metazoans, hedgehog consists of an amino-terminal signalling domain and carboxy-terminal hedgehog/intein (Hint) domain responsible for autocatalytically cleaving the protein. In one *M. brevicollis* hedgehog-like protein, a hedgehog N-terminal signalling domain is found at the N terminus of a large transmembrane protein that, instead of a Hint domain, includes von Willebrand A, cadherin, TNFR (tumor necrosis factor receptor), furin and EGF domains. Similar proteins are found in the sponge *Amphimedon queenslandica* and in the cnidarian *Nematostella vectensis*[36], revealing that the *M. brevicollis* genome captures an ancestral arrangement of protein domains rather than representing a lineage-specific domain-shuffling event. Another *M. brevicollis* hedgehog-like protein contains a Hint domain—a key region involved in the autocatalytic processing of hedgehog (Fig. 4b). The identification of a hedgehog-like gene in a choanoflagellate is not without precedent. A distinct Hint-domain-containing protein, named Hoglet, was identified in the distantly related *Monosiga ovata*[12], supporting the idea that isolated signalling components were present in the last common ancestor of choanoflagellates and metazoans.

Divergent use of phosphotyrosine signalling machinery

Phosphotyrosine (pTyr)-based signalling was considered unique to metazoans until its recent discovery in choanoflagellates[9,11]. The key domains involved in pTyr signalling are found in abundance in the *M. brevicollis* genome: tyrosine kinase domains that phosphorylate tyrosine (~120 occurrences), pTyr-specific phosphatases (PTP) that remove the phosphate modification (~30) and SH2 domains that bind pTyr-containing peptides (~80) (Supplementary Fig. 7). In contrast, these domains are rare in non-metazoans; for example, *S. cerevisiae* has no tyrosine kinase domains, only three PTP domains and a single SH2 domain. These findings support a model in which the full set of pTyr signalling machinery evolved before the separation of the choanoflagellate and metazoan lineages.

Although pTyr signalling machinery is present in metazoans and choanoflagellates, the mode of usage in *M. brevicollis* may be distinct from that in metazoans. A simple metric for the use of a particular domain is the range of domain types with which it is found in combination[37]. In the *M. brevicollis* genome, more than half of the observed pairwise domain combinations involving tyrosine kinase, PTP and SH2 domains are distinct from those seen in any metazoan genome (Fig. 5 and Supplementary Note 3.7). In contrast, for other sets of common signalling domains (those involved in phosphoserine/threonine (pSer/Thr), Ras–GTP and Rho–GTP signalling), most observed combinations are shared between *M. brevicollis* and metazoans. These observations are consistent with a simple model in which pSer/Thr, Ras–GTP and Rho–GTP signalling were fully elaborated before the branching of the choanoflagellate and metazoan lineages (consistent with the presence of these systems in other eukaryotes, including fungi, *Dictyostelium* and plants). In contrast, simple pTyr signalling may have emerged in the common ancestor and diverged radically between choanoflagellates and metazoans.

Streamlined transcriptional regulation

The core transcriptional apparatus of *M. brevicollis* is, in many ways, typical of most eukaryotes examined to date (Supplementary Table 10) including, for example, all 12 RNA polymerase II subunits and most of the transcription elongation factors (TFIIS, NELF, PAF, DSIF and P-TEFb, but not elongin). However, homologues of the largest subunit of TFIIF and several subunits of TFIIH are apparently lacking from the genome and the expressed-sequence-tag collection (Supplementary Fig. 8), reminiscent of the absence of several basal factors from the *Giardia lamblia* genome, suggesting alternative strategies for interacting with core promoter elements[38]. Similarly, only a limited number of general co-activators are identifiable in *M. brevicollis*, including the components of several chromatin-remodelling complexes (Supplementary Fig. 9 and Supplementary Note 3.8).

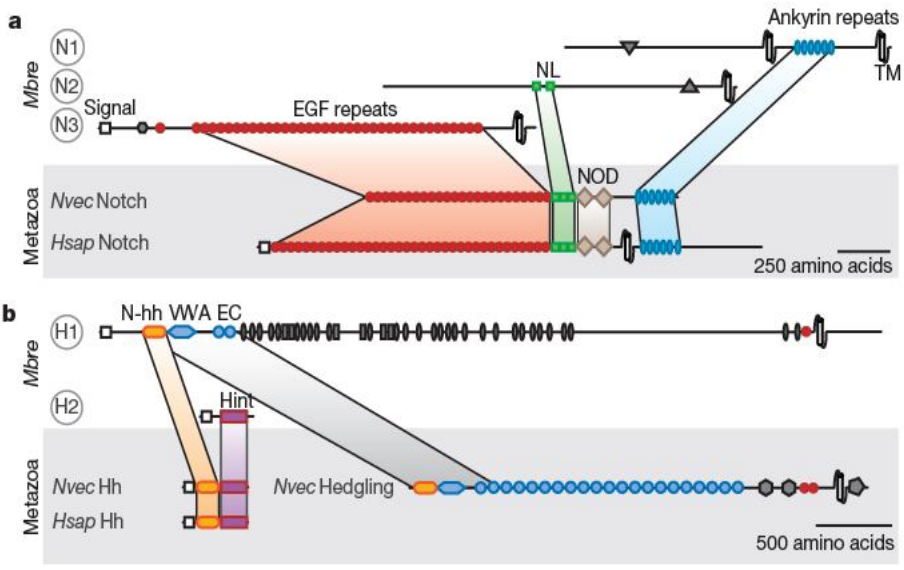

Figure 4 | Domain shuffling and the evolution of Notch and hedgehog. Analysis of the draft gene set reveals that *M. brevicollis* possesses proteins containing domains characteristic of metazoan Notch (**a**, N1–N3) and hedgehog (**b**, H1 and H2). Some of these protein domains were previously thought to be unique to metazoans. The presence of these domains in separate *M. brevicollis* proteins implicates domain shuffling in the evolution of Notch and Hedgehog. Hh, hedgehog; N-hh, hedgehog N-terminal signalling domain; Hint, hedgehog/intein domain; TM, transmembrane domain; VWA, von Willebrand A domain. See Supplementary Note 3.6 for protein accession numbers and Supplementary Fig. 6 for identification of all displayed protein domains. Species names follow the convention from Fig. 1.

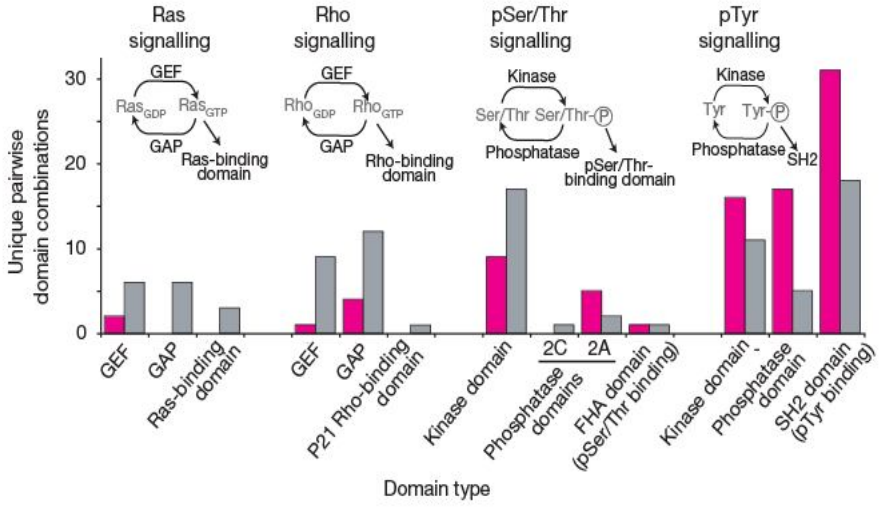

Figure 5 | Divergent usage of protein domains involved in pTyr-based signalling between *M. brevicollis* and metazoans. A metric for functional usage of a domain within a genome is the number of other domains with which it co-occurs in a single protein. Numbers of pairwise domain combinations are indicated for classes of signalling domains involved in Ras, Rho, pSer/Thr and pTyr signalling. In cases in which a domain combination occurs multiple times within an individual protein or genome, it is only counted once. All combinations observed in *M. brevicollis* are indicated either as those that are only observed in the *M. brevicollis* genome (magenta) or as those that are observed both in *M. brevicollis* and metazoan genomes (grey). pTyr signalling domains in *M. brevicollis* are unique in that most of their observed pairwise domain combinations are distinct from those observed in metazoans. GEF, guanine-nucleotide exchange factor; GAP, GTPase-activating protein.

Perhaps not surprisingly, *M. brevicollis* possesses members from most of the ubiquitous families of eukaryotic transcription factors (Supplementary Fig. 10). Most of the predicted transcription factors are zinc-coordinating; approximately 44% are C2H2-type zinc fingers. Eight proteins (5% of a total of 155 predicted transcription factors) are forkhead transcription factors, otherwise known only from metazoans and fungi.

The homeodomain transcription factors are an ancient protein family found in all known eukaryotes. At least two major superclasses of homeodomain proteins evolved before the origin of metazoans: those containing homeodomains of 60 amino acids (the 'typical', or non-TALE superclass), and those containing homeodomains of more than 63 amino acids (the TALE superclass)[39]. The *M. brevicollis* genome encodes only two homeodomain proteins, both of which group with the MEIS sub-class of TALE homeodomains (Supplementary Fig. 12). Apparently, genes encoding non-TALE homeodomain proteins have been lost in the lineage leading to *M. brevicollis*. *Bona fide* HOX class homeobox genes—a subclass of the non-TALE superclass—are absent from both *M. brevicollis* and the *Amphimedon queenslandica* (demosponge) genome sequence reads, indicating that this characteristic metazoan gene family probably emerged along the stem leading to eumetazoans[40].

M. brevicollis contains a subset of the transcription factor families previously thought to be specific to metazoans. Members of the metazoan p53, Myc and Sox/TCF families were identified, whereas many transcription factor families associated with metazoan patterning and development (ETS, HOX, NHR, POU and T-box) seem to be absent (Fig. 3).

Discussion

Choanoflagellates, sponges and other metazoans last shared a unicellular common ancestor in the late Precambrian period, more than 600 million years ago[41,42]. Although the origin of metazoans was a pivotal event in life's history, little is known about the genetic underpinnings of the requisite transition to multicellularity. Comparisons of modern genomes provide our most direct insights into the ancient genomic conditions from which metazoans emerged. By comparing choanoflagellate and metazoan genomes, we infer that their common ancestor had intron-rich genes, some of which encoded protein domains characteristically associated with cell adhesion and the ECM in animals.

In addition to containing protein domains associated with metazoan cell adhesion, *M. brevicollis* possesses a surprising abundance of tyrosine kinases and their downstream signalling targets. In contrast, components of most other intercellular signalling pathways, as well as many of the diverse transcription factors that comprise the developmental toolkit of modern animals, are absent. These presumably reached their modern form on the metazoan stem, although it is formally possible that they were in place much earlier and degenerated in the *M. brevicollis* lineage. Likewise, it is possible that the last common ancestor of choanoflagellates and metazoans had an early form of multicellularity that became more robust in metazoans and was lost in the choanoflagellate lineage. In any event, the evolutionary distance between choanoflagellates and metazoans is substantial, and evidently few, if any, intermediate lineages survived. There are, for example, no other known microbial eukaryotes that possess any of the eight developmental signalling pathways characteristic of metazoans.

The mechanism of invention of new genes on the metazoan stem, and their integration to create the emergent network of cell signalling and transcriptional regulation fundamental to metazoan biology, remains mysterious. Domain shuffling, which has frequently been proposed as an important mechanism for the evolution of metazoan multidomain proteins[43,44], is implicated by the presence of essential metazoan signalling domains in *M. brevicollis* that appear in unique combinations relative to animals. For pTyr-based signalling in particular, the marked divergence of domain combinations suggests that this mode of cellular interaction existed in a nascent form in the common choanoflagellate–metazoan ancestor, and was subsequently specialized and elaborated on in each lineage.

Given the limited transcription factor diversity in *M. brevicollis*, it is notable that the genome encodes representatives of the otherwise metazoan-specific p53, Myc and Sox/TCF transcription factor families. These transcription factors may have had early and critical roles in the evolution of metazoan ancestors by regulating the differential expression of genes to allow multiple cell types to exist in a single organism, and their study in choanoflagellates is a promising future direction.

The *M. brevicollis* sequence opens the door to genome-enabled studies of choanoflagellates, a diverse group of microbial eukaryotes that are important in their own right as bacterial predators in both marine and freshwater ecosystems. Although *M. brevicollis* is strictly unicellular, other choanoflagellates facultatively form colonies, and the modulation of these associations by cell signalling, adhesion, transcriptional regulation and environmental influences is poorly understood. An integrative approach that unites studies of choanoflagellate genomes, cell biology and ecology with the biogeochemistry of the Precambrian promises to reveal the intrinsic and extrinsic factors underlying metazoan origins.

METHODS SUMMARY

All analyses described were performed on Version 1.0 of the genome sequence. Details can be found in the Supplementary Information.

Separation of choanoflagellate and bacterial DNA. Using physical separation techniques combined with antibiotic treatments, a culture line with only a single bacterial food source, *Flavobacterium* sp., was developed. The GC content of *Flavobacterium* DNA (33%) is sufficiently different from that of *M. brevicollis* (55%) to allow separation over a CsCl gradient. *M. brevicollis* genomic DNA was

used to construct replicate libraries containing inserts of 2–3 kilobases (kb), 6–8 kb and 35–40 kb.

Genome sequencing, assembly and validation. The draft sequence of the *M. brevicollis* genome was generated from ~8.5-fold redundant paired-end whole-genome shotgun sequence coverage (Supplementary Information). Sequence data derived from six whole-genome shotgun libraries were assembled using release 2.9.2 of the whole-genome shotgun assembler Jazz. Completeness of the draft genome was assessed by capturing ~98.5% of sequenced expressed sequence tags.

Gene prediction and annotation. Gene models (9,196) were predicted and annotated using the Joint Genome Institute (JGI) Annotation Pipeline (Supplementary Information).

Intron analysis. Homologues of 473 highly conserved genes from *M. brevicollis* and representative eukaryotes were aligned to reveal the position and phylogenetic distribution of 1,989 highly reliable intron splice sites at 1,054 conserved positions. The evolutionary history of introns in orthologous genes was inferred using Dollo parsimony, Roy-Gilbert maximum likelihood and Csuros maximum likelihood[45–47].

Analysis of signalling, adhesion and transcription factor protein domains. Gene models containing metazoan signalling, adhesion and transcription factor domains were identified by text and protein domain ID searches of the JGI *M. brevicollis* genome portal, local BLAST searches within the *M.brevicollis* genome scaffolds, the online Pfam and SMART tools, and reciprocal BLAST searches in the NCBI non-redundant protein database (Supplementary Information).

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information The sequenced strain of *M. brevicollis* has been deposited at ATCC.org under accession number PRA-258. The genome assembly and annotation data are deposited at DBJ, EMBL and GenBank under the project accession ABFJ00000000. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to N.K. (nking@berkeley.edu) or D.R. (dsrokhsar@lbl.gov).

ARTICLES

Dual control of nuclear EIN3 by bifurcate MAPK cascades in C_2H_4 signalling

Sang-Dong Yoo[1], Young-Hee Cho[1], Guillaume Tena[1], Yan Xiong[1] & Jen Sheen[1]

A principal question in MAP kinase (MAPK/MPK) cascade signalling is how similar components dictate different specificity in the information-processing machineries from yeast to humans and plants. In *Arabidopsis*, how MPK3/6 modulates distinct outputs in diverse signal transduction pathways remains elusive. By combining systematic cellular and genetic screens, here we uncover a previously unexpected MKK9–MPK3/MPK6 cascade promoting ethylene-insensitive 3 (EIN3)-mediated transcription in ethylene signalling. The *mkk9* mutant exhibits a broad spectrum of moderate ethylene-insensitive phenotypes, and translocated MKK9 governs nuclear signalling downstream of receptors. Breaking a linear model and conventional MAPK signalling, ethylene inactivates the negative regulator constitutive triple response 1 (CTR1, a Raf-like MAPK kinase kinase (MAPKKK)) to activate the positive MKK9–MPK3/6 cascade. The bifurcate and antagonistic CTR1 and MKK9 pathways are both critical in determining ethylene-signalling specificity through two MAPK phosphorylation sites with opposite effects on EIN3 stability. The results suggest a new paradigm for linking intertwined MAPK cascades to control quantitative responses and specificity in signalling networks.

Ethylene (C_2H_4) was the first example of a gaseous signalling molecule in biological systems, discovered more than a century ago[1]. As a major plant hormone, it controls essential physiological processes, including: germination; root, shoot and flower development; stress, defence and glucose responses; fruit ripening; and senescence[1–5]. Extensive genetic analysis of ethylene signal transduction in *Arabidopsis* has established a linear pathway connecting five receptors[2–7] to a single negative regulator, CTR1 (ref. 8), and two key downstream positive components, EIN2 (ref. 9) and EIN3 (ref. 10). CTR1 encodes a putative Raf-like MAPKKK and interacts with the ethylene response 1 (ETR1) receptor, but its biochemical activity and molecular actions are unclear[8,11,12]. EIN3 and its closely related EIN3-LIKE1 (EIL1) are plant-specific nuclear transcription factors that initiate downstream transcriptional cascades for ethylene responses[10,13–15]. The identification of ethylene receptor, CTR1, EIN2 and EIN3 orthologues in diverse plant species[3] suggests the evolutionary conservation of ethylene signalling. However, it remains unknown how CTR1 functions as a MAPKKK to regulate downstream positive signalling components in the nucleus.

EIN3 interacts with two F-box proteins (EBF1 and EBF2) and is degraded by the 26S proteasome[5,16–19]. Ubiquitin/proteasome-dependent protein degradation mediated by specific F-box proteins of the conserved SCF (SKP1/cullin/F-box protein) E3 ubiquitin ligase complexes has emerged as a universal mechanism in response to multiple plant hormones, including auxin, gibberellin, abscisic acid, jasmonate and ethylene[20,21]. Understanding the distinct upstream signalling pathways that quantitatively control EIN3 and other transcription regulators through F-box protein-mediated degradation is a major interest in biology.

MAPK cascades are pivotal signalling modules controlling diverse signal transduction pathways in eukaryotes. A main question in MAPK cascade signalling has been how similar components control different biological responses[22–27]. In yeast and mammals, the specificity for distinct signalling pathways with shared components is largely determined by scaffolding proteins and specific MAPKs[25–27]. In plants, more complex roles of MAPKs have emerged. For instance, diverse hormones, stresses, microbial elicitors and developmental processes all activate the same conserved MPK3 and MPK6 in *Arabidopsis* and their orthologues in other plant species[22–24,28,29]. In ethylene responses, genetic and biochemical data present contradicting negative and positive roles of MAPK signalling[8,30–32]. It is unclear whether and how MAPK cascades are involved in the current linear genetic framework of the ethylene signal transduction pathway[3–5]. Adding to the complexity, *Arabidopsis* MPK6 is also shown to phosphorylate and stabilize ethylene biosynthetic enzymes, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS2/6), to promote ethylene synthesis[33]. Plant genomes encode the most extended families of putative MAPK cascade genes in eukaryotes[22,24]. Combining cellular and genetic screens with genomic information, we have initiated systematic and quantitative analyses of *Arabidopsis* MAPK cascades. Here we report the unexpected discovery of an MKK9–MPK3/6 cascade that positively modulates nuclear EIN3 stability. Remarkably, CTR1 acts as an unconventional MAPKKK, blocking MKK9–MPK3/MPK6 activation and simultaneously enhancing EIN3 degradation by distinct MPK phosphorylation. Our results illuminate new molecular mechanisms for the control of transcription-factor stability by intertwined MAPK cascades to achieve quantitative signalling specificity in eukaryotes.

Screens for MPKs and MKKs in ethylene signalling

To assess critically the role and molecular identity of MAPK cascades in ethylene signalling, we have initiated an integrative approach by combining molecular, genomic, biochemical and genetic tools. To increase the sensitivity of the MAPK screen, we used *ctr1-1* (*ctr1*) protoplasts with no endogenous CTR1 activity in the background[15,30,31,34–36]. Unexpectedly, constitutively active CTR1 (CTR1a) clearly repressed MPK1, MPK3 and MPK6 activities without altering protein expression based on an immunocomplex MAPK assay[34–36] (Fig. 1a). The result raised a possibility that the putative CTR1 MAPKKK could negatively regulate downstream MAPK activities, which could play a positive role in ethylene signalling[22,30,31]. Because little *MPK1* but abundant *MPK3* and *MPK6* transcripts were detected

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in protoplasts and leaves (Supplementary Fig. 1a), MPK3 and MPK6 are probably the physiological MAPKs in ethylene signalling.

We next attempted to identify the MAPKKKs (MKKs) that could activate MPK3/6 in ethylene signalling. There are ten putative *MKK* genes in the *Arabidopsis* genome[22,24], and all except *MKK8* and *MKK10* are expressed in mesophyll cells. We performed a primary screen to identify constitutively active MKK (MKKa) that could activate co-expressed MPK3/6 in wild-type (WT) protoplasts. The MKKa constructs were generated by site-directed mutagenesis to convert threonine/serine to aspartic acid/glutamic acid mimicking phosphorylation[36] and activation by upstream MAPKKKs. In addition to MKK4a and MKK5a[36], MKK7a and MKK9a activated MPK3/6 based on an immunocomplex MAPK assay (Fig. 1b). Interestingly, overexpressing WT MKK7 and MKK9 specifically and preferentially activated the epitope-tagged MPK3/6 (Fig. 1c) or endogenous MAPKs corresponding to the size of MPK3/6 (Supplementary Fig. 1b) in *ctr1* protoplasts lacking the key negative regulator CTR1. Wild-type MKK4 and MKK5 did not activate MPK3/6 in WT or *ctr1* protoplasts (Fig. 1c and Supplementary Fig. 1c). Although MKK4/5 and MKK7/9 could all activate MPK3/6, they appeared to have differential functions in MAPK signalling networks. The CTR1 MAPKKK could act unconventionally as a direct or indirect negative regulator of the MKK7/9–MPK3/6 cascade.

MKK9 activates EIN3-mediated transcription in *ctr1*

To connect this novel and positive MAPK cascade to the key and specific nuclear transcription factor EIN3 in ethylene signalling, we conducted a second MKK screen using an early ethylene-responsive luciferase (LUC) reporter directly targeted by EIN3 for transcription activation[15]. The reporter carrying four synthetic copies of the defined EIN3 binding site (*EBS*)[10,13,15] was highly and specifically activated by EIN3 co-expression within 3 h in the transient assay (Fig. 2a). The short timeframe ensured the activation of only the earliest and direct EIN3 target genes and avoided secondary responses. The promoter of an early ethylene response gene *ERF5*, but not other promoters inducible by flg22 (*WRKY29*)[36], abscisic acid (*RD29A*)[35] and auxin (*GH3*)[34,35], was also activated by co-expressed EIN3, confirming the specificity of the assay (Fig. 2a). Furthermore, *EBS-LUC* responded to ethylene in WT but not in ethylene-insensitive *ein2* protoplasts lacking EIN3 (ref. 17) (Fig. 2b). Correlated with the CTR1a repression of MPK3/6 activities (Fig. 1a), CTR1a but not inactive InCTR1^{K579M} reduced *EBS-LUC* activity in *ctr1* protoplasts (Supplementary Fig. 1d).

We performed a systematic screen based on quantitative *EBS-LUC* response to identify WT MKKs that could enhance ethylene signalling in *ctr1* protoplasts. In agreement with the specific MPK3/6 activation in *ctr1* protoplasts (Fig. 1c), expression of WT MKK7 or MKK9 uniquely enhanced *EBS-LUC* activity (Fig. 2c). The constitutively active MKK7a or MKK9a displayed even greater ability to activate *EBS-LUC* (Fig. 2d). *Arabidopsis* MKK4a/5a and tobacco NtMEK^{DD} activate MPK6, which phosphorylates and enhances cytosolic ACS2/6 stability to elevate ethylene levels[33]. The differential MKK4a/5a–MPK3/6 and MKK7a/9a–MPK3/6 effect on the early ethylene reporter gene activation in *ctr1* suggests signalling specificity downstream of ethylene perception, probably in the nucleus. Because the *ctr1* mutant phenotype is not as severe as *etr1 ers1* or *ebf1 ebf2* double mutants[16,18,19,37,38] and can further respond to ethylene[39], this positive MAPK cascade may represent an additional regulatory pathway between the receptors and the F-box proteins. Although MKK7 and MKK9 are highly homologous and showed similar activities in the MAPK and reporter activation, the steady-state *MKK9* transcript level was much higher in protoplasts and leaves (Supplementary Fig. 1e). Thus, MKK9 is likely to play a predominant role in ethylene signalling in mesophyll cells. Consistently, the activated MKK9 but not the MKK9 kinase mutant immunoprecipitated from protoplasts could directly phosphorylate *in vitro* GST–MPK3/6

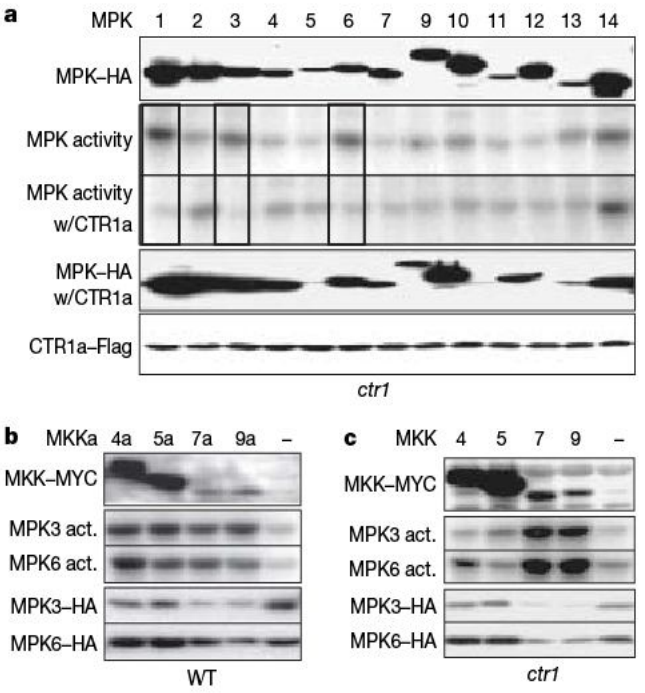

Figure 1 | Cell-based genetic screens for specific MPKs and MKKs in ethylene signalling. **a**, Specific MAPKs are activated in the *ctr1* protoplasts but inactivated by CTR1a. MAPK activity and protein expression (MPK–HA or CTR1a–Flag) are shown. The experiments were repeated twice. **b**, Constitutively active MKK4a, MKK5a, MKK7a and MKK9a activate MPK3 and MPK6 in WT protoplasts. **c**, Transient expression of WT MKK7 or MKK9 preferentially increases MPK3 and MPK6 activity (act.) in *ctr1* protoplasts. Empty vector (−) was used as a DNA transfection control. Protein expression (MKK–MYC or MPK–HA) is shown.

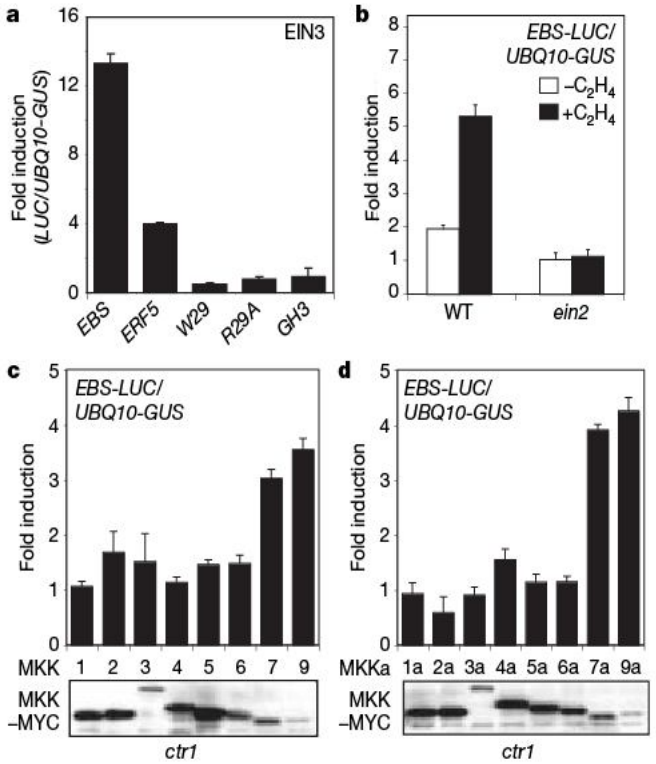

Figure 2 | Specific MKKs modulate EIN3-dependent transcription. **a**, EIN3 specifically induces *EBS-LUC* and *ERF5-LUC* activities in WT protoplasts. **b**, C_2H_4 (10 p.p.m.) induces *EBS-LUC* activity in WT but not in *ein2-1* protoplasts. **c**, WT MKK7 or MKK9 specifically enhances *EBS-LUC* activities in *ctr1* protoplasts. **d**, Constitutively active MKK7a or MKK9a further enhances *EBS-LUC* activities in *ctr1* protoplasts. Protein expression (MKK–MYC) is shown. Error bars, s.d. ($n = 3$). All experiments were repeated three times with similar results.

produced from *Escherichia coli* without autophosphorylation[36] (Supplementary Fig. 2).

mkk9 exhibits diverse ethylene-insensitive phenotypes

To obtain genetic evidence to independently evaluate the surprising and specific positive role of MKK9 in ethylene signalling, we performed quantitative ethylene response screens of *mkk* loss-of-function mutants collected from various transferred DNA (T-DNA) insertion resources[40,41] (Supplementary Figs 3–5 and Supplementary Table 1). The WT and various mutant plants were grown under the same condition and harvested at the same time to minimize plant-to-plant variations. As shown in Fig. 3a, only the etiolated *mkk9* seedlings displayed ethylene insensitivity in hypocotyl similar to *ein3* in the presence of 0.5–1 μM of ACC[3–5,10,14,19]. The ethylene-insensitive phenotype was similar in two distinct *mkk9* alleles from different sources (Supplementary Fig. 5). We further examined the specific role of MKK9 in ethylene signalling by comparing the phenotypes of *mkk9-1* and *ein3* in five additional assays[10,15,42,43]. Both *mkk9* and *ein3* displayed similar hypersensitivity to glucose and salt (Fig. 3b, c). The link between ethylene and glucose or salt sensitivity was further supported by the hypersensitive phenotypes of other stronger ethylene-insensitive mutants, such as *etr1* and *ein2* (refs 42, 43). Both *mkk9* and *ein3* leaves showed little chlorophyll degradation induced by ACC in the dark[10] (Supplementary Fig. 6a), and were relatively resistant to ethylene inhibition of leaf and petiole elongation under light[10] (Supplementary Fig. 6b). Importantly, ethylene activation of immediate early marker genes, *ERF1* and *ERF5*, was abolished in *mkk9* and *ein3* leaves (Fig. 3d). These marker genes are direct EIN3 targets[13,15] (Fig. 2a) and are specific indicators of early ethylene signalling. The inability of *mkk9* to respond to exogenous ethylene in early transcription activation ruled out the possibility that the *mkk9* phenotypes were due to lower endogenous ethylene biosynthesis. The results also provided important evidence to uncouple the MAPK regulation of ethylene signalling from ethylene synthesis.

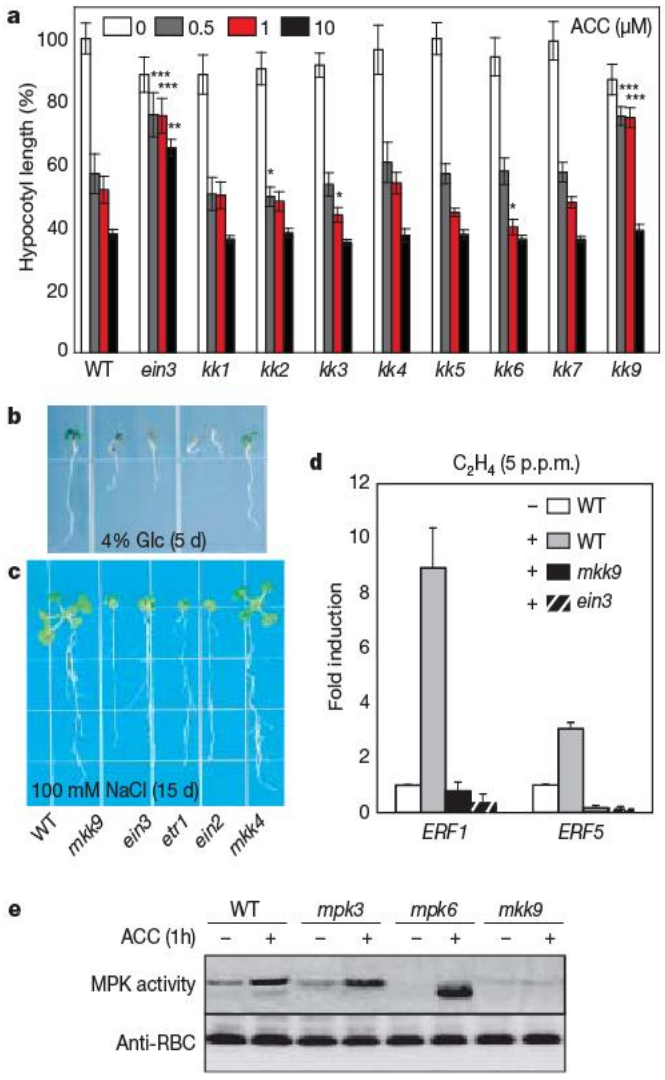

Figure 3 | The loss-of-function *mkk9* mutant displays diverse ethylene-insensitive phenotypes. **a**, The *mkk9* and *ein3* seedlings exhibit ethylene insensitivity in hypocotyl elongation at 0.5–1 μM ACC. Error bars, s.d. ($n = 20$). Asterisks indicate differences between WT and mutant with statistical significance at $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ (*t*-test). **b**, **c**, The seedlings of *mkk9* and other ethylene-insensitive mutants show glucose (**b**) and salt (**c**) hypersensitivity. **d**, Immediate early gene induction by C_2H_4 (5 p.p.m.) is defective in *mkk9* and *ein3* leaves measured by qRT–PCR. Error bars, s.d. ($n = 3$). **e**, Endogenous MAPKs activated by ACC in WT, *mpk3* and *mpk6* leaves. Leaves were treated with 200 μM ACC for 1 h[34–36]. Rubisco (anti-RBC), loading control.

To assess whether the ethylene insensitivity of *mkk9* is due to the loss of the MKK9-dependent MPK3/6 activation in ethylene signalling, ACC activation of MAPK activity was measured in detached leaves after assay optimization (Supplementary Fig. 7a). Two endogenous MAPKs were activated in WT but not in *mkk9* after ACC feeding through the petiole to minimize mechanical stress during the treatment (Fig. 3e and Supplementary Fig. 7a). Maximal MAPK activities were detected at 1 h because of the time required for ACC uptake and conversion to ethylene *in vivo*. We used the loss-of-function *mpk3* and *mpk6* single mutants[33] to verify that the ACC- and MKK9-dependent activation of MAPKs were indeed MPK3 and MPK6 (Fig. 3e). Our results are consistent with previous independent studies showing that ethylene or ACC activated MAPK[30,31]. Interestingly, MPK6 activation by ACC was elevated in *mpk3*, whereas the activation of MPK3 was greatly enhanced in *mpk6*. The results suggest that MPK3 and MPK6 play redundant roles and can compensate for the loss of each other in ethylene signalling (Fig. 3e). Using the virus-induced gene silence (VIGS) method[44] to bypass embryo lethality[29], we showed that the inductions of *ERF1* and *ERF5* were significantly diminished in the *mpk3 mpk6* double mutants (Supplementary Fig. 7b). The combined genetic analyses of *mkk9* and *mpk3 mpk6* mutant plants provide compelling *in vivo* evidence to support their specific roles in ethylene signalling.

MKK9a promotes constitutive ethylene signalling

To substantiate the role of MKK9 in ethylene signalling, we generated transgenic *Arabidopsis* plants expressing the constitutively active MKK9a in WT or different ethylene-insensitive mutants. Transgenic lines were first screened for equal *MKK9a* transgene expression in the WT, *etr1* and *ein2* background (Supplementary Fig. 8a) without overt cell death before the phenotypic analyses. MKK9a transgenic plants showed constitutive ethylene phenotypes in the WT background (Fig. 4a, b). Similar ethylene phenotypes have been observed in multiple receptor mutants[7,37,38] or the double mutant *ebf1 ebf2* accumulating EIN3 (refs 16, 18, 19). Moreover, *ERF1* and *ERF5* were highly activated by MKK9a in the transgenic *Arabidopsis* (Fig. 4c).

To place MKK9a in the genetically established ethylene-signalling pathway[2–5], we analysed the effects of MKK9a in the ethylene-insensitive *etr1* and *ein2* mutants. The MKK9a phenotypes, including etiolated seedling responses and gene activation, were not blocked by *etr1* with little ethylene perception (Fig. 4c, d). The results were consistent with the insensitivity of MKK9a seedling phenotypes to an ethylene receptor antagonist Ag^+ (ref. 17) (Fig. 4a), suggesting that MKK9a acted downstream of the ethylene receptors but not simply enhancing ethylene synthesis. In contrast, the MKK9a phenotypes were diminished in the *ein2* mutant (Fig. 4c, d). A straightforward genetic model would place MKK9 upstream of EIN2, analogous to the common interpretation of the *ctr1 ein2* double mutant[2–5]. However, it is equally possible that the reduced effects of MKK9a could be explained by the absence of EIN3 as the MKK9 cascade

target in the *ein2* mutant[17], in which EIN3 overexpression could overcome its defect[10]. The gain-of-function analyses of MKK9a in transgenic plants provided additional *in vivo* evidence to support its role in ethylene signalling. The *mkk9* mutant was complemented (Fig. 4e, f) with WT *MKK9* (Supplementary Fig. 8b). Although the etiolated *ctr1* and the *ctr1 mkk9* double mutant seedlings looked similar, the elongated light-grown *ctr1* seedling phenotype[9] was diminished in the *ctr1 mkk9* double mutant in the absence or presence of ACC (Supplementary Fig. 9), supporting the idea that MKK9 acted downstream of CTR1.

Dual phosphorylation modulates EIN3 stability

We next examined how MKK9–MPK3/6 cascades might regulate EIN3 in ethylene signalling[15–18]. Interestingly, an MKK-specific inhibitor, U0126, blocked EIN3 accumulation induced by ACC (Fig. 5a), and phosphatase caused EIN3 band shift, suggesting EIN3 phosphorylation in seedlings (Fig. 5b). Constantly, the constitutively active MKK9a–GFP but not MKK4a–GFP preferentially localized in the nucleus (Fig. 5c), whereas MPK6 distributed in both the cytoplasm and nucleus (Supplementary Fig. 10a). Furthermore, the WT MKK9–GFP was translocated into the nucleus in response to ACC in WT but not in *etr1* protoplasts (Fig. 5d), even though total MKK9–GFP protein levels were similar in WT and *etr1* (Supplementary Fig. 10b). Significantly, the immunoprecipitated MPK3 or MPK6 activated by MKK9a in protoplasts could directly phosphorylate EIN3 *in vitro* (Fig. 5e). These results suggest that direct protein phosphorylation by MKK9–MPK3/6 in the nucleus may be a key step for EIN3 protein stabilization and ethylene signalling.

To map the MAPK phosphorylation site(s) in EIN3 directly, we combined computational analyses and targeted mutagenesis, which were followed by comprehensive cellular and transgenic analyses *in vivo*. Two robust and comprehensive motif search algorithms, Eukaryotic Linear Motif (ELM)[45] and Scansite 2.0 (ref. 46), were used to search for putative MAPK phosphorylation sites and the conserved

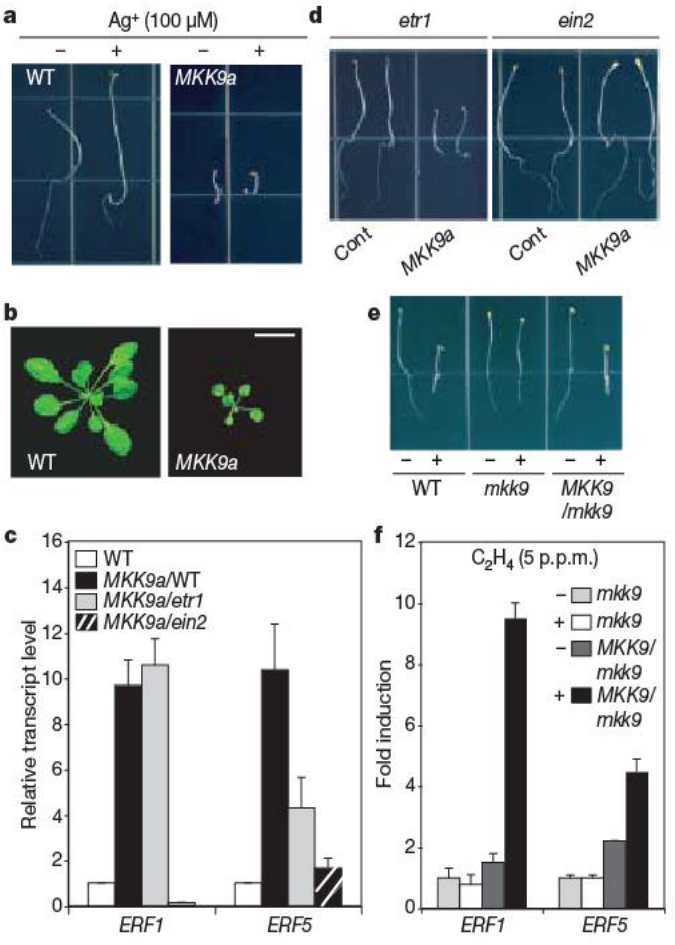

Figure 4 | Constitutively active *MKK9a* confers ethylene signalling. **a**, MKK9a confers constitutive ethylene responses. Ag+ (100 μM). **b**, Adult 28-day-old *MKK9a* transgenic plants exhibit a constitutive ethylene phenotype. Scale bar, 10 mm. **c**, MKK9a promotes immediate early ethylene gene expression measured by qRT–PCR. Error bars, s.d. ($n = 3$). **d**, MKK9a action is blocked in *ein2* but not in *etr1* seedlings. Control (Cont) *etr1* or *ein2* seedlings do not carry *MKK9a*. **e**, The *mkk9* etiolated seedlings are complemented by *MKK9* (*MKK9/mkk9*). **f**, Induction of ethylene response genes measured by qRT–PCR is restored in the *MKK9*-complemented lines (*MKK9/mkk9*). Error bars, s.d. ($n = 3$).

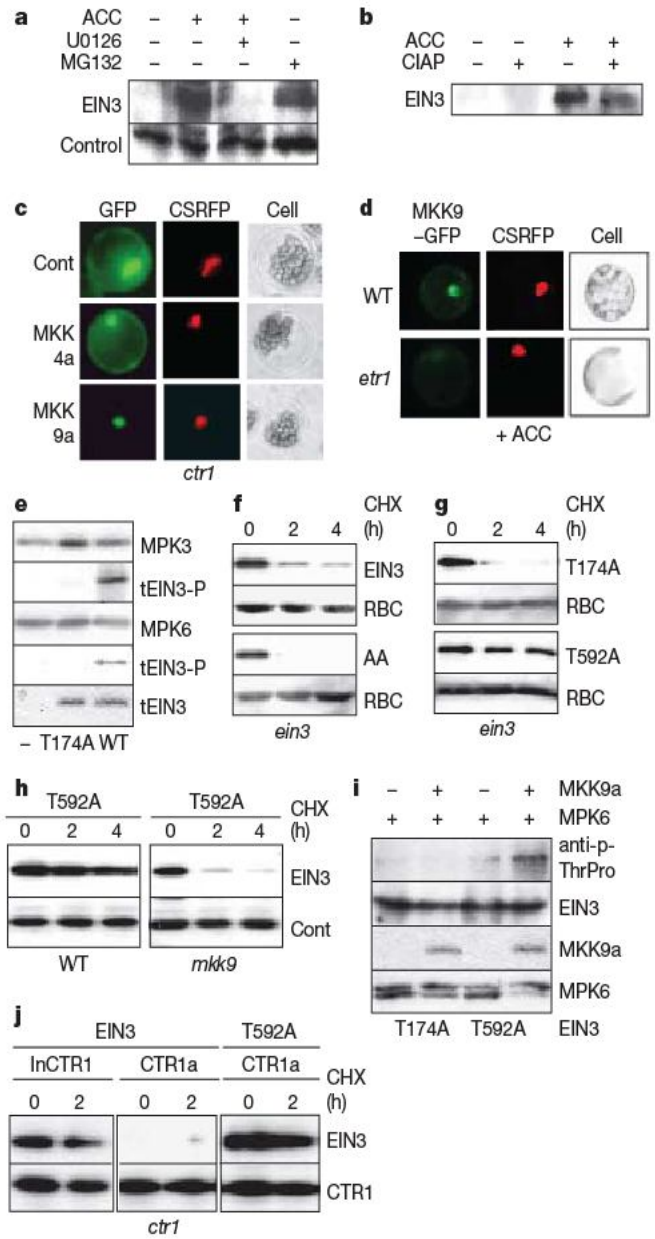

Figure 5 | Dual phosphorylation regulates EIN3 stability. **a**, EIN3 regulation in seedlings. Tubulin (control). **b**, EIN3 phosphorylated *in vivo*. The EIN3 protein mobility shifts without (−) or with (+) phosphatase (CIAP). **c**, MKK9a–GFP is localized in the nucleus. Nuclear RFP marker[36] (CSRFP). MKK4a–GFP is localized in both the cytoplasm and nucleus as GFP (cont). **d**, MKK9–GFP is translocated into the nucleus by ACC treatment in WT but not in *etr1*. **e**, MPK3 and MPK6 directly phosphorylate T174 in a truncated EIN3 protein *in vitro*. tEIN3 (ref. 15), amino acids 141–352. MPK3/6 (anti-HA) and tEIN3 (Coomassie blue staining) protein levels are shown. **f**, The $EIN3^{AA}$ mutant shows enhanced protein degradation. RBC as control. **g**, Opposite effects of two phosphorylation sites on EIN3 stability. **h**, Enhanced $EIN3^{T592A}$ stability requires MKK9. **i**, T174 in EIN3 is phosphorylated *in vivo* by MKK9a-activated MPK6. Immunoprecipitated EIN3 was examined with a p-Thr-Pro-specific antibody. **j**, Active CTR1a promotes EIN3 degradation through T592 phosphorylation. Inactive InCTR1 is a control.

docking sites for MAPK in EIN3. There were two predictable MAPK phosphorylation sites (174T175P and 592T593P), each coupled with a putative conserved-docking motif nearby, conserved in EIN3 and EIL1 (refs 14, 19). Remarkably, the predicted MAPK conserved-docking motif (amino acids 244–252) near 174T was mutated in the *ein3-3* mutant (K244D) that diminished EIN3 function[10]. In an *in vitro* assay, the T174A mutation prevented EIN3 phosphorylation by activated MPK3/6 (Fig. 5e).

To determine the *in vivo* function of T174 and T592 in ethylene signalling, we generated the $EIN3^{T174A/T592A}$ ($EIN3^{AA}$) mutant, and the stability of EIN3 and $EIN3^{AA}$ proteins was monitored after cycloheximide (CHX) treatment in the transfected *ein3* protoplasts. The non-phosphorylatable $EIN3^{AA}$ mutant always degraded faster than the WT EIN3 protein (Fig. 5f). Unexpectedly, when the individual phosphorylation site mutants ($EIN3^{T174A}$ or $EIN3^{T592A}$) were tested with the same assay, we discovered their opposite effects on EIN3 stability. Compared with EIN3 (Fig. 5f), the T174A mutation enhanced EIN3 degradation whereas the T592A mutation enhanced EIN3 stability (Fig. 5g). It was clear that the endogenous MKK9 was required to stabilize $EIN3^{T592A}$ (Fig. 5h) in WT protoplasts with some level of constitutive ethylene signalling (Fig. 2b) that could phosphorylate T174. We also showed that the activated MPK6 phosphorylated $EIN3^{T592A}$ but not $EIN3^{T174A}$ *in vivo* by a specific antibody that recognized a phospho-Thr-Pro motif in protoplasts (Fig. 5i). Finally, CTR1a abolished EIN3 but not $EIN3^{T592A}$, supporting a direct link between T592 phosphorylation and degradation by the CTR1 pathway (Fig. 5j). As CTR1–GFP did not accumulate in the nucleus, the results indicated that the CTR1 pathway probably acted through MAPKs (Fig. 1a) to phosphorylate T592 and promotes EIN3 degradation in the nucleus. We proposed a model that the MKK9 cascade phosphorylates T174 to promote EIN3 stability, whereas T592 is phosphorylated by an MAPK pathway mediated by CTR1 to promote EIN3 degradation.

To test our model in plants further, we generated and examined *ein3* transgenic plants complemented with WT EIN3, $EIN3^{AA}$, $EIN3^{T174A}$ and $EIN3^{T592A}$. For informative comparisons and to avoid the dominant effect of EIN3 overexpression[10], we selected multiple transgenic lines for each construct with similar levels of *EIN3* expression as the endogenous *EIN3* in WT (Supplementary Fig. 11). Consistent with the results obtained using the cellular assays, $EIN3^{AA}$ and $EIN3^{T174A}$ transgenic lines were insensitive to saturating ACC (Fig. 6a). On the contrary, the $EIN3^{T592A}$ lines exhibited ACC hypersensitivity. EIN3 protein accumulation patterns reflected the transgenic phenotypes (Fig. 6a). Detailed quantification of ACC dose responses confirmed that the transgenic $EIN3^{T174A}$ lines were more insensitive to ACC and the transgenic $EIN3^{T592A}$ lines were hypersensitive to ACC (Fig. 6b). The fact that the $EIN3^{T592A}$ lines only displayed weak constitutive ethylene phenotypes in the absence of ACC strongly supports the model that EIN3 phosphorylation on T174 by MKK9–MPK3/6 is activated by ethylene signalling and is critical for its stabilization even in the absence of T592 phosphorylation, which enhanced EIN3 degradation (Fig. 5g, j). Both the inhibition of CTR1 and activation of MKK9 are required for ethylene signalling specificity. The data explain why *mkk9* exhibited moderate ethylene-insensitive phenotypes (Fig. 3) and escaped classical mutant screens, as only one of the bifurcate pathways (Fig. 6c) was inactive. The stronger constitutive ethylene signalling phenotypes in *ctr1* are partly attributed to the activation or de-repression of the MKK9–MPK3/6 cascade revealed in *ctr1* protoplasts (Figs 1 and 2).

Discussion

Signalling specificity is fundamental to proper function of regulatory pathways in eukaryotic cells. A common theme in the evolutionarily conserved MAPK cascade signalling is the use of the same components in different signal transduction pathways. Many strategies can maximize the functions of a limited set of MAPK cascade modules for different biological responses and processes, including different partners in distinct cell types, subcellular compartmentalization, response amplitude and duration, temporal separation, and the use of scaffolding proteins[22,25–27]. In *Arabidopsis*, the confounding effects of MAPKs on both ethylene synthesis and signalling, as well as other stress and defence responses and diverse developmental processes,

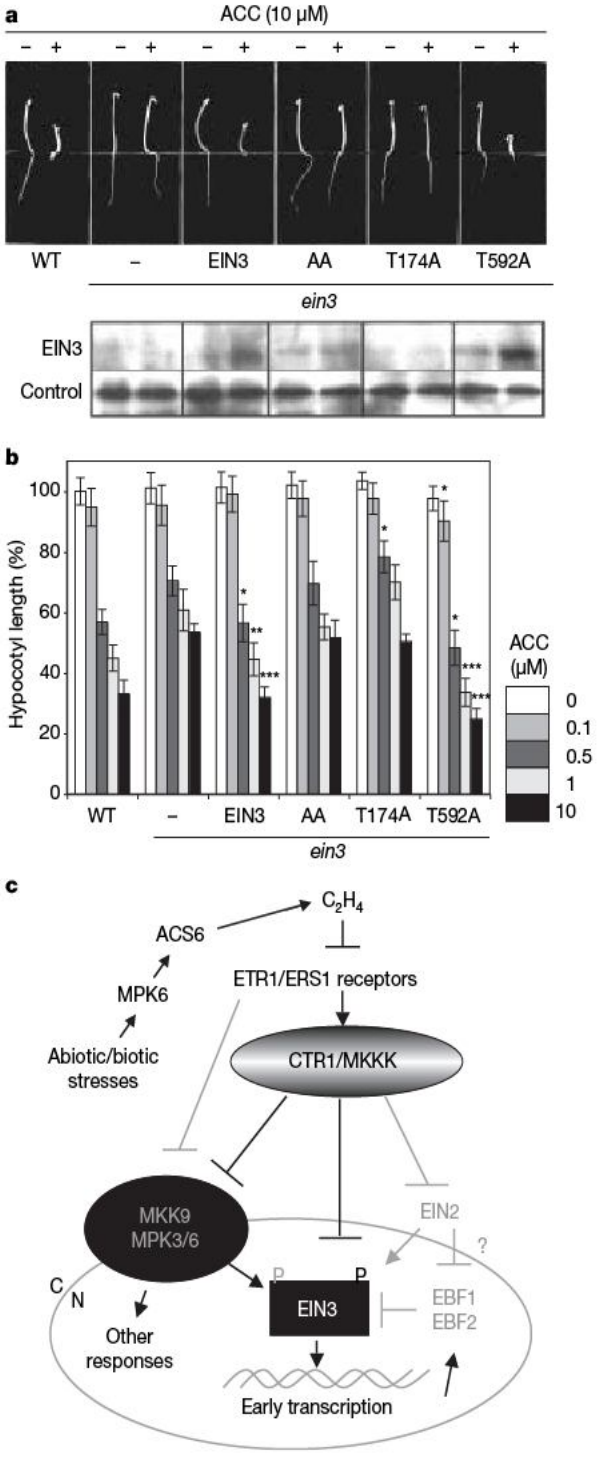

Figure 6 | Analysis of EIN3 mutants in transgenic *ein3* plants. **a**, Ethylene sensitivity assay. EIN3 protein accumulation in the transgenic lines is shown. Histone (control). **b**, Quantitative analyses of hypocotyl elongation of transgenic lines. Error bar, s.d. ($n = 20$). Asterisks indicate differences between *ein3* and transgenic *ein3* plants with statistical significance at $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ (*t*-test). **c**, Model of the bifurcate MAPK cascades in ethylene signalling. The two EIN3 phosphorylation sites (P) with opposite functions are marked. Without ethylene, CTR1 directly or indirectly inactivates MKK9–MPK3/6 and probably activates downstream MAPKs to phosphorylate T592 to promote EIN3 degradation. Ethylene inactivates CTR1 for MKK9–MPK3/6 activation and T174 phosphorylation to stabilize EIN3. Arrow and blunt ends indicate positive and negative regulations, respectively. ACS, ACC synthase; MKKK, MAPKKK; C, cytoplasm; N, nucleus.

impose great challenges in defining MAPK signalling specificity[22-24,28–36]. We provide compelling evidence for the identification of a novel MKK9–MPK3/6 cascade that phosphorylates and stabilizes EIN3 in ethylene signalling. The MKK9–MPK3/6 cascade targets EIN3 in the nucleus to distinguish temporarily and spatially its positive ethylene signalling function from the MKK4/5–MPK6 activity on ACS6 for ethylene biosynthesis in the cytoplasm[33]. Further biochemical and genetic analyses reveal the surprising opposite functions of the dual EIN3 phosphorylation sites, T174 for stabilization and T592 for degradation. The simultaneous activation of the MKK9 cascade and the inhibition of the CTR1 pathway specify quantitative control of EIN3 levels and EIN3-mediated transcription. CTR1 as a unique MAPKKK appears to control bifurcate and antagonistic MAPK cascades targeted to the same key nuclear transcription factor in ethylene signalling (Fig. 6c). The flexible and quantitative mechanism can facilitate the molecular connections in a complex signal network modulated by hormonal, metabolic and environmental signals to serve an adjustable and adaptive lifestyle characteristic of plants. The involvement of CTR1 in auxin, gibberellin and glucose responses[15,47] suggests that the CTR1 pathway may integrate other signals, and can be involved in phosphorylation and control of other transcription factors in distinct signalling pathways without the MKK9 partnership. It is also possible that the MKK9–MPK3/6 cascade can be modulated by signals other than ethylene and can participate in multiple stress and defence responses independent of CTR1 (Fig. 6c). Similar systematic and integrated approaches used in this study may be applicable for the identification of the subtle and redundant MAPK cascade components presumably acting downstream of CTR1. How CTR1 regulates EIN2 and inhibits MKK9–MPK3/6, whether other MAPKKKs activate MKK9, and whether EBF1 and EBF2 are also regulated by MAPK cascades, remain to be determined (Fig. 6c). There are over 100 putative MAPK cascade genes in *Arabidopsis*, and many are conserved in agriculturally important plants[24,48]. It will be interesting to elucidate how the positive and negative functions of MAPK cascades interact in the signalling networks modulated by a myriad of internal and external signals to govern essential biological processes[22–27].

METHODS SUMMARY

Protoplast transient assays. Transient expression and MAPK activity assays were performed in *Arabidopsis* mesophyll protoplasts as described[15,35,36,49]. Subcellular localizations of MKK4a–GFP, MKK9a–GFP and ACC-treated MKK9–GFP were observed in transfected protoplasts by fluorescent microscopy[15,36,49]. Protein stability assays were conducted using WT and mutant EIN3 proteins as described[15]. Transfected *ein3* protoplasts were incubated for 4 h before treatment with 10 μM CHX to stop *de novo* protein synthesis. EIN3 protein degradation was visualized by immunoblot analysis.

MPK and MKK screens. The MKK9–MPK3/6 module was identified by sensitized screens with epitope-tagged MPKs and MKKs by using immunocomplex MAPK activity[35,36] and the ethylene-specific *EBS-LUC* reporter[15] assays in *ctr1* protoplasts[8,49].

The *mkk* mutant screens. The loss-of-function *mkk* mutants were identified by standard methods[40,41] and screened for the ethylene-specific hypocotyl response using etiolated seedlings free of other stresses[10,14].

Gene expression analysis. Quantitative polymerase chain reaction with reverse transcription (qRT–PCR) was used with gene-specific primers for *ERF1* and *ERF5* in WT, mutants and transgenic lines.

Transgenic plants. The *mkk9-1* mutant was complemented with a WT *MKK9* genomic construct. The gain-of-function *MKK9a* transgenic lines were generated in WT, *etr1-1* and *ein2-1* background and used for epistasis analyses. The WT and various EIN3 mutants were introduced into *ein3* plants. Multiple transgenic lines were selected with equal transgene expression for analyses of ethylene response.

EIN3 *in vivo* phosphorylation. We examined EIN3 accumulation and phosphorylation induced by ACC (100 μM) with etiolated seedlings in the absence or presence of an MKK inhibitor (U0126, 10 μM), a proteasome inhibitor (MG132, 50 μM) and/or a general phosphatase (calf intestine alkaline phosphatase) using protein blot analyses with a specific EIN3 antibody[15]. We detected *in vivo* EIN3 phosphorylation by activated MPK6 in protoplasts with protein blot analysis using a p-Thr-Pro-specific antibody and the EIN3 phosphorylation mutants $EIN3^{T174A}$ and $EIN3^{T592A}$.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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ARTICLES

Cohesin mediates transcriptional insulation by CCCTC-binding factor

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Cohesin complexes mediate sister-chromatid cohesion in dividing cells but may also contribute to gene regulation in postmitotic cells. How cohesin regulates gene expression is not known. Here we describe cohesin-binding sites in the human genome and show that most of these are associated with the CCCTC-binding factor (CTCF), a zinc-finger protein required for transcriptional insulation. CTCF is dispensable for cohesin loading onto DNA, but is needed to enrich cohesin at specific binding sites. Cohesin enables CTCF to insulate promoters from distant enhancers and controls transcription at the *H19/IGF2* (insulin-like growth factor 2) locus. This role of cohesin seems to be independent of its role in cohesion. We propose that cohesin functions as a transcriptional insulator, and speculate that subtle deficiencies in this function contribute to 'cohesinopathies' such as Cornelia de Lange syndrome.

In proliferating cells, cohesin complexes physically connect replicated DNA molecules ('sister chromatids') from S phase until the subsequent anaphase of mitosis or meiosis. This sister-chromatid cohesion is essential for chromosome segregation and for DNA damage repair. Cohesin is composed of four core subunits, called SMC1, SMC3, SCC1 (also known as MDC1 and RAD21) and SCC3 (also known as SA2 and STAG2)[1–3]. These proteins have been proposed to mediate cohesion by embracing sister chromatids as a ring[4].

The essential role of cohesin in cohesion is well established, but evidence obtained in yeast and different animal species implies that cohesin also contributes to gene regulation, chromatin structure and development[5–13]. Furthermore, certain human diseases have been linked to hypomorphic mutations in cohesin and in proteins that regulate cohesin. Cornelia de Lange syndrome (CdLS) is characterized by growth and mental retardation, craniofacial anomalies and microcephaly. This disease can be caused by mutations in a protein that is required to load cohesin onto DNA, called SCC2 (also known as NIPBL and delangin), or by mutations in SMC1 or SMC3 (refs 14–17). Roberts/SC phocomelia syndrome (RBS/SC, OMIM 26900) is a related disease that has been linked to mutations in ESCO2, a protein implicated in the establishment of cohesion[18,19]. Surprisingly, most of these mutations do not cause obvious defects in cell proliferation, implying that the resulting developmental abnormalities are not caused by defects in cohesion but reflect a distinct, yet unknown, function of cohesion proteins.

Cohesin is expressed in differentiated postmitotic cells

In vertebrates, cohesin binds to chromatin at the end of mitosis, long before cohesion is established in the next cell cycle[3,20,21]. This suggests that cohesin may also have a function on unreplicated DNA, independent of its role in cohesion. To explore this possibility, we first tested if cohesin is also expressed in postmitotic cells, which lack cohesion. Immunoblotting experiments identified SCC1 and SMC1 in numerous mouse tissues, including brain (Fig. 1a). SMC3 antibodies immunoprecipitated cohesin complexes from brain extracts (Fig. 1b), and by immunofluorescence microscopy (IFM), SCC1 staining was observed in the nuclei of neurons (Fig. 1c and Supplementary Fig. 1). PDS5B, a protein that is associated with cohesin, has recently also been detected in mouse neurons[13]. Cohesin is therefore

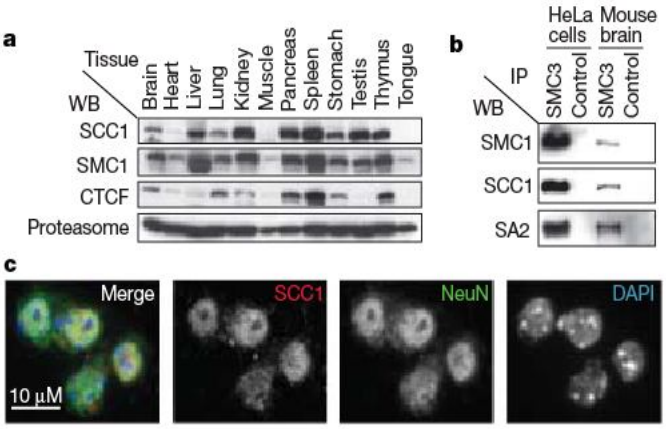

Figure 1 | Cohesin is expressed in postmitotic cells. a, Mouse tissue extracts were analysed for cohesin and CTCF expression by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting with the indicated antibodies. WB, western blot. **b**, SMC3 immunoprecipitates (IP) obtained from mouse brain and HeLa interphase extracts were analysed by SDS–PAGE and immunoblotting with the indicated antibodies. **c**, Frozen thin sections from mouse brain cortex were co-stained for SCC1, the neuronal marker NeuN and DNA (4,6-diamidino-2-phenylindole, DAPI).

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expressed in differentiated postmitotic cells, consistent with the possibility that cohesin performs functions in addition to cohesion.

Identification of cohesin-binding sites in the human genome

We next identified cohesin-binding sites in the human genome by chromatin immunoprecipitation (ChIP). We initially used Affymetrix oligonucleotide tiling arrays designed by the ENCODE project[22] (22-base pair (bp) resolution, representing 1% of the genome). In HeLa cells synchronized in the G2 phase, SCC1 antibodies identified 167 sites with high statistical confidence. Most of these were also detected with antibodies to SMC3 and SA2, but not with unrelated antibodies, such as anti-p53 (Fig. 2a, Supplementary Fig. 2 and Supplementary Table 1). Most if not all of these sites are cohesin-specific, because depleting SCC1 by RNA interference (RNAi) eliminated or reduced the signals in SMC3 ChIP-chip experiments (Fig. 2a). We confirmed 27 cohesin sites by analysing SCC1 and SMC3 ChIP samples with quantitative polymerase chain reactions (qPCRs), whereas no enrichment was found at 9 control sites (Fig. 2b and Supplementary Table 2).

By ChIP-chip, we found that most SCC1 sites (82%) were identical in the G1 phase and G2 phase, indicating that cohesin binds to the same sites independent of cohesion. No specific sites could be detected in mitotic cells, consistent with the finding that most cohesin dissociates from chromosome arms in prophase[3,20,21]. Most SCC1 sites were also found in diploid hTERT RPE-1 and immortalized B cells; we detected more sites in these cells than in HeLa cells (Supplementary Table 1). Different cell lines may therefore contain both common and unique cohesin sites.

To investigate the genome-wide distribution of cohesin, we analysed SCC1 ChIP samples on Affymetrix arrays that represent all non-repetitive elements of the human genome with 35 bp resolution. We identified 8,811 sites with high confidence (P-value $< 5.0 \times 10^{-8}$). Most of these are located in intergenic regions (49%), in introns (35%) or within 5 kilobases (kb) upstream or downstream of genes (13%; Supplementary Fig. 3). The latter sites are over-represented compared to their frequency in the genome (Fig. 2c). The average distance between two sites is 340 kb and ranges from 480 bp to 35.6 megabases (Mb).

For yeast, it has been proposed that cohesin can be moved along DNA by the transcription machinery[23,24]; consistent with this possibility, more than 80% of cohesin sites on chromosome arms are regions of convergent transcription[25]. In the human genome, however, more than 3,000 cohesin sites are found in introns, many of which are actively transcribed in HeLa cells. In humans, transcription can therefore not remove cohesin permanently from genes.

Cohesin co-localizes with CTCF in the human genome

We noticed that cohesin is enriched at several sites to which the transcriptional insulator protein CTCF[26] binds, such as the *H19* imprinting control region (ICR) and the β-globin locus control region (LCR). To assess whether cohesin and CTCF co-localize also at other sites, we mapped CTCF sites in HeLa, hTERT RPE-1 and B cells by ChIP-chip on ENCODE arrays (Fig. 2a and Supplementary Table 1). Many CTCF sites were identical to SCC1 sites. For example, in HeLa cells in G2 phase, 165 out of 167 SCC1 sites are also bound by CTCF. Accordingly, the consensus sequences for SCC1 and CTCF sites are very similar (Fig. 2d). Similar results were obtained with genome-wide arrays (Supplementary Fig. 4). We identified 13,894 CTCF sites, 7,813 of which were identical to SCC1 sites, corresponding to 89% of all SCC1 sites. CTCF sites at which we did not detect SCC1 by ChIP-chip may nevertheless be bound by cohesin, because we also detected cohesin at these sites by ChIP-qPCR (Supplementary Fig. 5e and Supplementary Table 2). Recently, independent studies have identified CTCF sites by ChIP-chip, ChIP-sequencing and bioinformatics[27–29]. Although different cells were used, more than 60% of our CTCF sites were also identified in these studies.

We could not co-immunoprecipitate cohesin and CTCF from HeLa extracts (data not shown), indicating that the soluble forms of these proteins are not stably bound to each other. As predicted by ChIP-chip, cohesin and CTCF antibodies yielded similar IFM patterns (Supplementary Fig. 6). Both showed nuclear staining in interphase and cytoplasmic staining from prometaphase until anaphase. Contrary to earlier findings[30], we could detect little if any CTCF on mitotic chromosomes by IFM, ChIP-qPCR and immunoblotting (Supplementary Figs 6 and 7). Like cohesin, most CTCF therefore dissociates from chromosomes in mitosis and rebinds in telophase.

CTCF is required for the positioning of cohesin on DNA

Given the high degree of co-localization, we wondered whether CTCF is required for enrichment of cohesin at specific sites. To test this, we analysed SCC1 and SMC3 by ChIP-qPCR in CTCF-depleted HeLa cells. As a control, we analysed SCC2-depleted cells in which cohesin loading onto DNA is reduced[31]. As predicted, the abundance of cohesin sites was reduced after SCC2 depletion, but remarkably the same was true after CTCF depletion (Supplementary Fig. 5a, b). When we analysed SCC1 ChIP samples from CTCF-depleted cells on ENCODE arrays, we could only detect 77 out of 167 cohesin sites (Fig. 2a; Supplementary Table 1). We used cells in the G2 phase for these experiments, ruling out the possibility that cohesin binding was reduced because cells accumulated in mitosis. CTCF is therefore required for enrichment of cohesin at its proper binding sites. In contrast, in ChIP-qPCR experiments we also found a 50% reduction in CTCF binding after cohesin depletion (Supplementary Fig. 5c, d). Cohesin might therefore also contribute to CTCF positioning.

We next investigated if CTCF is required for loading of cohesin onto DNA, as is the case for SCC2. Immunoblot experiments indicated that this is not the case, because we observed similar SCC1 signals in chromatin pellets in control and CTCF-depleted HeLa and hTERT RPE cells (Supplementary Fig. 8a and data not shown). Quantitative IFM (qIFM) confirmed that chromatin-bound cohesin levels were not reduced after CTCF depletion, and CTCF on chromatin was also not reduced by SCC1 or SCC2 depletion (Supplementary Fig. 8b–d). Cohesin and CTCF can therefore associate with DNA independently of each other. Accordingly, we did not observe major cohesion defects in CTCF-depleted cells (data not shown). These data suggest that cohesin is distributed more broadly on DNA in the absence of CTCF, and may therefore not be detectable by ChIP-chip.

Cohesin is required for the insulator function of CTCF

To test if cohesin is required for CTCF function, we measured transcriptional changes in SCC1- and CTCF-depleted HeLa cells by DNA-chip technology. In G2 cells, we identified 194 transcripts that were upregulated and 90 transcripts that were downregulated after both SCC1 and CTCF depletion (Supplementary Tables 3 and 4). Interestingly, genes within 25 kb of cohesin sites had a higher tendency to be upregulated than downregulated (Fig. 2e), consistent with an insulator function for cohesin/CTCF sites[32]. Cohesin and CTCF may therefore regulate genes near their binding sites in similar ways.

Some CTCF sites are known to function as transcriptional insulators. We therefore tested if cohesin depletion influences the ability of CTCF sites to insulate a gene from a distant enhancer. We first analysed the *H19*ICR (Fig. 3a), which normally controls transcription at the *H19*/*IGF2* locus[33,34]. On a reporter plasmid, the *H19*ICR can also block a simian virus 40 enhancer from activating the *H19* promoter, which drives expression of firefly luciferase[35] (Fig. 3b); this effect depends on CTCF[35]. We used this system to test whether transcriptional insulation also requires cohesin.

By ChIP-qPCR, we observed binding of CTCF and cohesin to the luciferase reporter plasmids when these were transiently transfected into HeLa cells (Fig. 3c). As reported[35], CTCF depletion increased luciferase activity in cell lysates, indicative of increased luciferase

expression; remarkably, depletion of SCC1 and SMC3 had very similar effects (Fig. 3d). Luciferase activity was also decreased by depletion of the cohesin-loading factor SCC4, but not by depletion of SCC2 by RNAi, perhaps owing to incomplete SCC2 depletion (Supplementary Fig. 9). Depletion of the cohesion establishment factor sororin[36] had no effect. When we used a plasmid in which the ICR between the luciferase gene and the simian virus 40 enhancer had been mutated so that CTCF binding is lost[35], luciferase activity increased only insignificantly after CTCF, SCC1 or SMC3 depletion (Fig. 3b, d). The effects of cohesin and CTCF depletion therefore

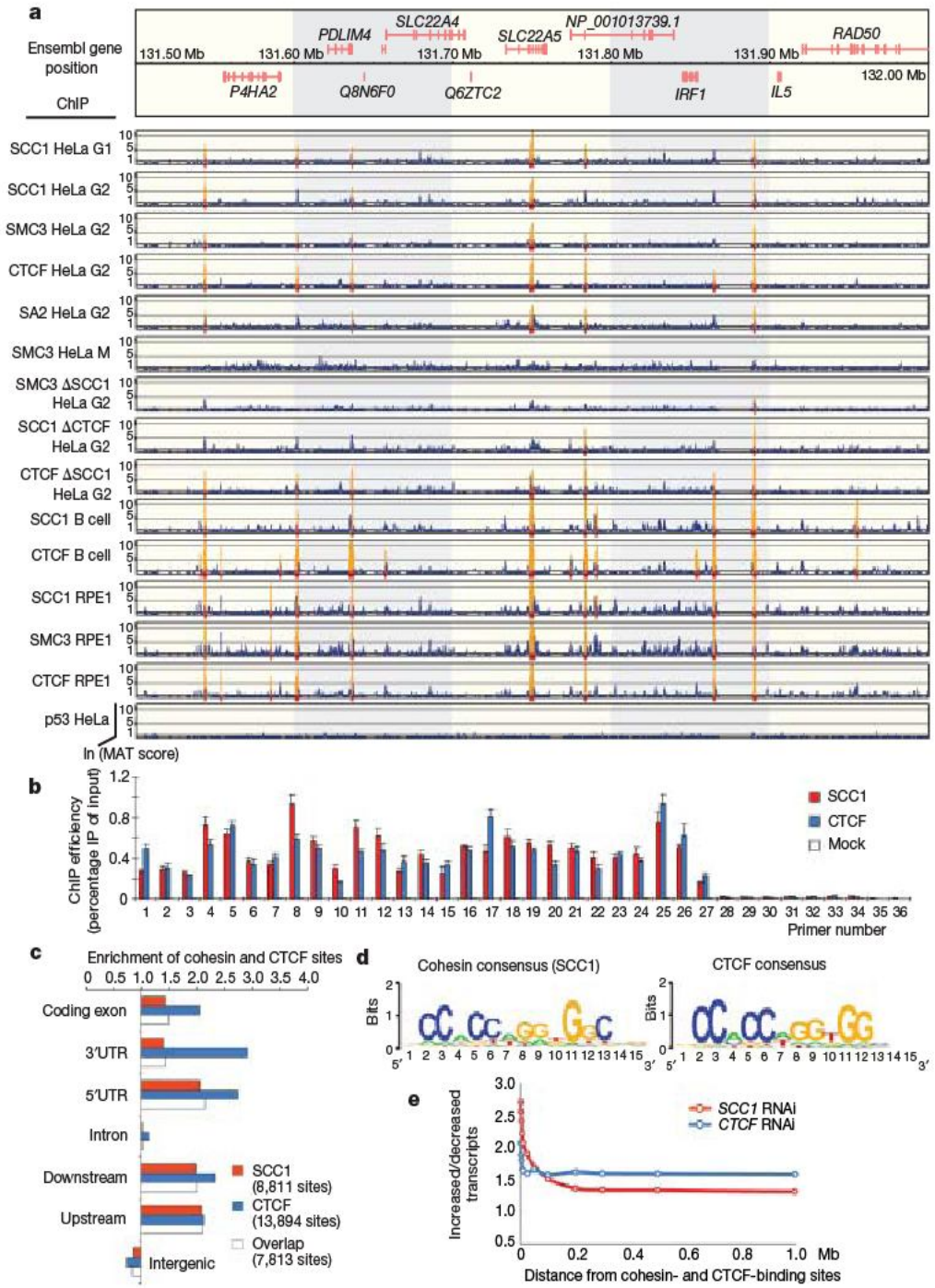

Figure 2 | Identification of cohesin- and CTCF-binding sites in the human genome. **a**, Examples of ChIP-chip data obtained with ENCODE arrays (position 131.5–132 Mb of human chromosome 5). Vertical axes show the MAT score (log scale), which reflects the fold-enrichment of the ChIP-chip samples. Regions in which signals were significantly enriched are coloured in orange with red flags. Cells were enriched in G1 phase or G2 phase by double-thymidine arrest/release (dTAR), and in mitosis (M) by dTAR followed by 2 h nocodazole treatment and shake-off. ΔSCC1 and ΔCTCF, cells depleted by RNAi of SCC1 and CTCF, respectively. All samples were prepared from interphase cells unless indicated otherwise. **b**, SCC1 and CTCF ChIP-qPCR data obtained with primers (Supplementary Table 2) designed for 27 SCC1-positive and 9 negative sites identified by ChIP-chip. In all cases, positive sites were enriched more than 25-fold compared to negative sites (mean of $n = 3$; error bars ± s.d.). **c**, Frequencies of cohesin- and CTCF-binding sites in non-repetitive regions of the human genome (approximately 50% of the entire genome): intergenic regions, introns, exons, and 5′ and 3′ untranslated regions (UTRs) of Ensemble genes are shown, as well as regions 5 kb upstream and downstream of 5′ UTRs and 3′ UTRs, respectively. The number of sites in these regions was normalized against the frequencies of these regions in the genome and displayed as fold enrichment. **d**, DNA consensus sequences for SCC1- and CTCF-binding, derived from ENCODE array data with the MEME tool[50]. The height of each letter represents the relative frequency of nucleotides at different positions in the consensus. More than 70% of 362 CTCF sites and 75% of 168 SCC1 sites analysed contain these motifs. **e**, Relationships between the distance of SCC1- and CTCF-binding sites from genes and transcriptional changes induced by RNAi-mediated depletion of SCC1 and CTCF. Transcriptional analyses were carried out using MAS (microarray analysis software, provided by Affymetrix), and significantly increased and decreased genes were extracted using the default threshold. The ratio of numbers of increased/decreased genes (y-axis) was plotted against the distance from each binding site (x-axis).

depend on a functional ICR. We used synchronized G2 cells in all of these experiments, ruling out indirect cell-cycle effects. We therefore conclude that the insulator function of the *H19* ICR requires cohesin binding to DNA, but not the establishment of cohesion.

We also tested if cohesin is required for the function of the *HS4* (DNase hypersensitive site 4) insulator, which is located in the β-globin LCR[37]. *HS4* can block the effect of enhancers on distant promoters in a CTCF-dependent manner, and *HS4* can act as a barrier to chromosomal position effects[32,38]. To test if the insulator function of *HS4* depends on cohesin, we used HeLa cell lines in which the expression levels of tandemly oriented marker cDNAs are increased by the presence of chicken *HS4* insulators[39]. When SCC1- and CTCF-depleted cells were analysed by IFM and immunoblotting, marker cDNA expression was reduced, whereas endogenous proteins were unaffected (Supplementary Figs 10 and 11). Cohesin may therefore also contribute to the insulator function of *HS4*.

Cohesin controls transcription at the imprinted *H19/IGF2* locus

The *H19* ICR ensures that *H19* is only transcribed from the maternal allele, whereas the neighbouring *IGF2* gene is only transcribed from the paternal allele (Fig. 4a; reviewed in ref. 40). This imprinting depends on CTCF, which can bind to the *H19* ICR on the maternal allele where it prevents *IGF2* activation from a distant enhancer, which instead activates *H19*. In contrast, CTCF binding to the paternal allele is blocked by methylation on CpG sequences. As a consequence, the distal enhancer activates *IGF2* but cannot stimulate *H19* transcription[33,34].

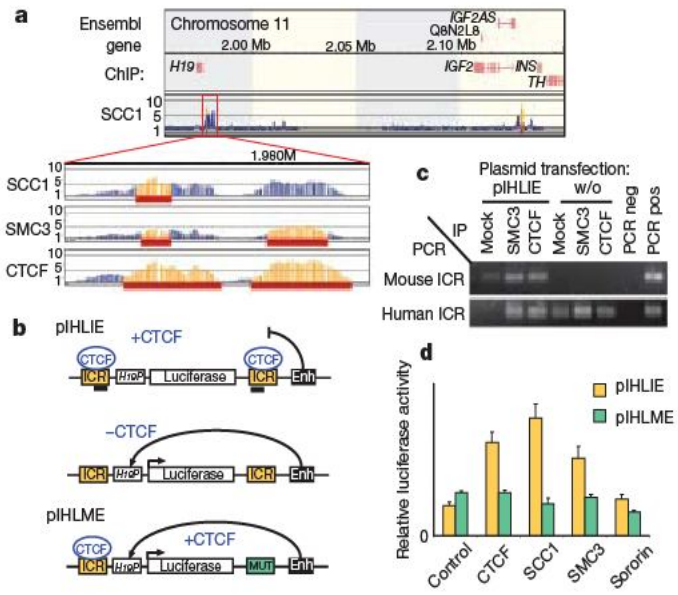

Figure 3 | Cohesin is required for the insulator function of the *H19* ICR. **a**, Cohesin co-localizes with CTCF at the *H19/IGF2* locus. SCC1 ChIP-chip data are shown for the entire locus together with an enlarged view of SCC1, SMC3 and CTCF ChIP-chip data for the *H19* ICR. **b**, Schematic representation of the reporter constructs pIHLIE and pIHLME that were used in enhancer-blocking assays. On pIHLIE, CTCF binding to the ICR represses luciferase expression by blocking enhancer access to the promoter. In pIHLME, *H19* ICR nucleotides required for CTCF binding have been mutated[35]. Enh, enhancer; *H19P*, mouse *H19* promoter; MUT, ICR mutated in a way that CTCF cannot bind. The black line shows the fragment amplified by PCR. **c**, SMC3 and CTCF ChIP samples obtained from HeLa cells transfected with pIHLIE were analysed by PCR with primers specific for the mouse *H19* ICR (located on pIHLIE) or the endogenous human *H19* ICR. neg, negative control (neither ChIP nor whole cell extract included); pos, positive control (whole extract of transfected cells included in PCR); w/o, without transfection. **d**, HeLa cells were depleted by RNAi of the indicated proteins and transfected with a Renilla luciferase control plasmid together with either pIHLIE or pIHLME, which contain firefly luciferase cDNAs. After synchronization in the G2 phase, the ratios of firefly versus Renilla luciferase activities were determined in the cell lysate and normalized against control RNAi (mean of $n = 3$; error bars, ± s.d.).

To investigate whether cohesin is required for imprinting, we first tested if cohesin, like CTCF, is specifically bound to the maternal *H19* ICR, which is located on human chromosome 11. To address this, we used mouse cells that carry either the maternal or the paternal allele of human chromosome 11 (ref. 41). Chromosome-specific fluorescence *in situ* hybridization (FISH) confirmed the presence of a single copy of human chromosome 11 in these hybrid cells (Fig. 4b), and PCR with reverse transcription (RT–PCR) indicated that specific transcripts such as *KCNQ1OT1*, *KCNQ1* and *H19* are still expressed from human chromosome 11 in an imprinted fashion[41,42] (Fig. 4c and data not shown). The endogenous mouse *H19* ICR could be isolated with CTCF and SMC3 antibodies from both cell lines by ChIP-qPCR, but the same antibodies could isolate the human *H19* ICR only from cells carrying the maternal human chromosome 11 (Fig. 4d). Like CTCF, cohesin is therefore specifically bound to the maternal allele of the *H19* ICR. We confirmed this notion by re-ChIP experiments in which we isolated DNA fragments first with CTCF antibodies, eluted the ChIP samples from the antibody beads and then re-immunoprecipitated with SCC1 antibodies, or *vice versa*. For all tested cohesin sites, including the human *H19* ICR, we found that cohesin and CTCF are bound to the same DNA molecules (Fig. 4e).

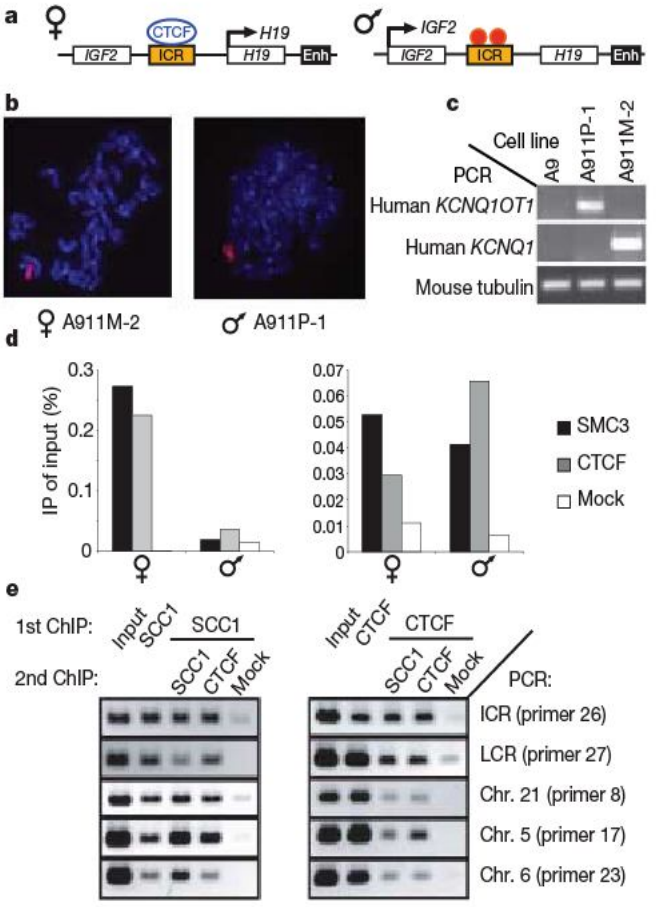

Figure 4 | Cohesin co-localizes with CTCF on the maternal allele of the *H19* ICR. **a**, Schematic representation of the *H9/IGF2* locus and its transcriptional regulation. Circles represent DNA methylation. **b**, Detection of human chromosome 11 in mouse–human hybrid cells by FISH. A911M-2 and A911P-1 cells contain a maternal and paternal copy of human chromosome 11, respectively[41]. **c**, Hybrid cells as in **b** were analysed by RT–PCR for the presence of paternal (*KCNQ1OT1*) or maternal-specific (*KCNQ1*) transcripts encoded by human chromosome 11. **d**, SMC3 and CTCF ChIP samples obtained from hybrid cells as in **b** were analysed by qPCR with primers specific for the human (primer 96, left) or the mouse (primer MmH19, right) *H19* ICR (data representative of several independent experiments are shown). **e**, Re-ChIP assays to test co-localization of cohesin and CTCF on the same DNA molecules. ChIP experiments were either performed first with SCC1 antibodies and then analysed by re-ChIP with CTCF antibodies, or *vice versa*. All samples were analysed by PCR with the indicated primers. Chr., chromosome.

To test if cohesin is required for imprinting, we analysed the levels of *H19* and *IGF2* transcripts in cohesin- and CTCF-depleted HeLa cells. In control cells, both *H19* and *IGF2* transcripts could be detected, although *IGF2* only at low levels. After CTCF depletion, *H19* transcripts were reduced, and *IGF2* transcript levels were increased, implying that *IGF2* and *H19* are also imprinted in HeLa cells in a CTCF-dependent manner (Fig. 5a). Remarkably, SCC1 depletion had a very similar effect. In contrast, neither CTCF nor SCC1 depletion altered the transcript levels of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, Fig. 5a). The changes in *H19* and *IGF2* transcription were not caused by cell-cycle effects because we used G2-synchronized cells for these experiments (Supplementary Fig. 9a, b). These data suggest that cohesin is required for imprinting at the *H19*/*IGF2* locus. Importantly, we observed that SCC1 and CTCF depletion altered *H19* and *IGF2* transcript levels in a very similar way in cells that were synchronized in the G1 phase (Fig. 5b, c). Because sister-chromatid cohesion does not exist in the G1 phase, the role of cohesin in controlling *H19* and *IGF2* transcription seems to be independent of cohesin's function in cohesion (see also Supplementary Fig. 12).

Discussion

Because hypomorphic mutations in genes required for cohesion can cause defects in chromatin organization, transcription and development, it has long been suspected that cohesin has functions in addition to its role in sister-chromatid cohesion[5–13]. However, it has been difficult to exclude the possibility that these phenotypes are indirect consequences of subtle or rare cohesion defects, and it has remained unknown what the molecular basis of cohesion-independent cohesin functions could be. Our results indicate that cohesin has an important role at CTCF-binding sites, which may function as transcriptional insulators or boundary elements in vertebrate genomes. Hypomorphic mutations in cohesion genes may therefore cause defects in insulators and boundaries that could lead to transcriptional and developmental abnormalities. Recent studies using a mouse model of the cohesinopathy CdLS have indeed revealed transcriptional changes that are consistent with this possibility (A. Lander, personal communication).

Our observation that cohesin depletion alters *H19* and *IGF2* transcription not only in the G2 phase but also in the G1 phase, where cohesion does not exist, implies that some of the functions of cohesin are indeed independent of its role in cohesion. This notion is supported by our finding that cohesin, like CTCF, is widely expressed in mammalian tissues, most of which are predominantly composed of postmitotic cells. However, this does not exclude the possibility that sister-chromatid cohesion also affects transcription, which could explain why mutations in the putative cohesion establishment factor ESCO2 can cause RBS/SC syndromes.

The hypothesis that cohesin has important transcriptional roles during the G1 phase and in postmitotic cells can also provide a potential explanation for why vertebrate cells remove the bulk of cohesin from chromosome arms in prophase and reload it onto DNA in telophase[3,20,21] instead of destroying most cohesin in metaphase, as occurs in yeast[43]. Because the protease separase preferentially cleaves chromosome-bound cohesin[21], the prophase pathway may spare cohesin from destruction in metaphase so that cohesin can perform cohesion-independent functions immediately after mitosis.

In the future it will be interesting to understand how cohesin contributes to transcriptional insulation at the mechanistic level. Cohesin complexes can physically connect two distinct DNA molecules when they mediate cohesion between sister chromatids[4]. It is therefore conceivable that cohesin can also physically connect different sites on one DNA molecule, thereby creating DNA loops that could control enhancer–promoter interactions topologically[44,45]. Alternatively, cohesin could physically block the spreading of transcription factors, chromatin-remodelling enzymes and heterochromatin proteins on DNA.

Cohesin has been highly conserved in eukaryotes and related complexes exist in bacteria[4], but CTCF has only been described in vertebrates and *Drosophila*[46]. Cohesin might therefore have adopted an insulator function late in evolution, or cohesin might perform insulator functions without CTCF in lower eukaryotes. Along these lines, it is conceivable that the main function of CTCF in mammalian genomes is to define binding sites for cohesin, and that cohesin is the molecule that structures DNA in a way that causes insulator and boundary effects.

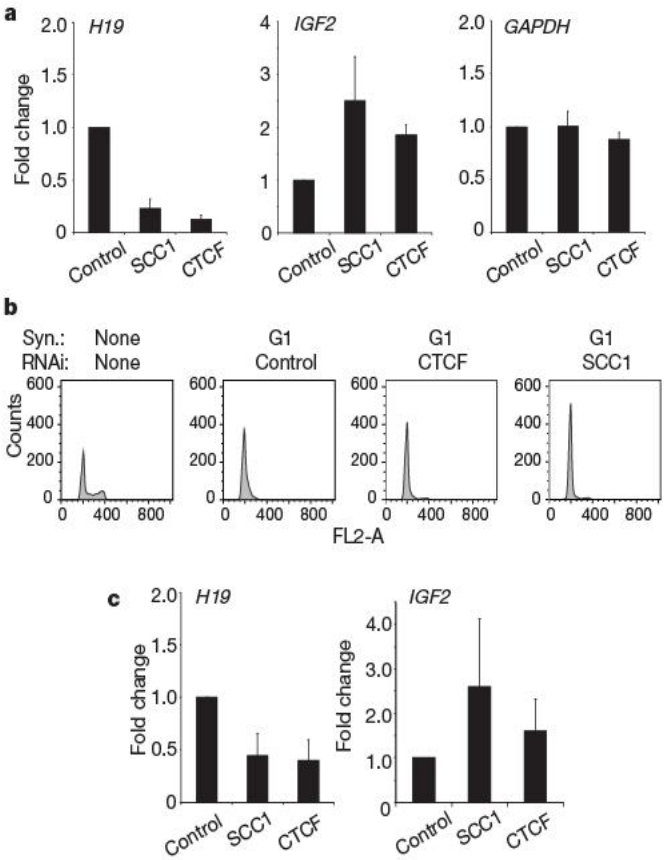

Figure 5 | Cohesin controls transcription at the *H19/IGF2* locus. **a**, RT–qPCR analysis of *H19*, *IGF2*, *GAPDH* and actin transcripts in SCC1- and CTCF-depleted HeLa cells synchronized in G2 phase. The transcript levels were normalized first to control RNAi values and second to actin levels (mean of $n = 3$ for *H19* and *IGF2*, $n = 2$ for *GAPDH*; error bars, ± s.d.). **b**, Fluorescence-activated cell sorting (FACS) analysis of cells that were enriched in G1 phase by dTAR, and removal of mitotic cells by shake-off. Syn., synchronization; FL2-A, signal intensity of the propidium iodine stain. **c**, RT–qPCR analysis as in **a** of cells synchronized in G1 phase. Transcript levels were normalized to GAPDH (mean of $n = 3$; error bars, ± s.d.).

METHODS SUMMARY

Chromatin immunoprecipitation was performed as described[47].

The immunoprecipitated DNA (ChIP) and a control for the non-enriched DNA (whole-cell extract, WCE) were amplified by *in vitro* transcription, labelled by biotin and hybridized to high-density oligonucleotide arrays (Affymetrix) as described in ref. 48. After scanning and data extraction, ChIP and WCE signals for each of the tiling arrays were normalized by the model-based analysis of tiling-arrays algorithm (MAT)[49]. The MAT score was calculated and mapped to genomic positions in the human genome assembly human genome (Hg) 18 (NCBI Build 36), and significantly enriched regions were selected using the MAT score. False detection rates, calculated by the MAT program, were less than 2% for all experiments (Supplementary Fig. 13).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Information Microarray data presented in this article have been deposited in the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) under the accession number GSE9613. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to K.S. (kshirahi@bio.titech.ac.jp) or J.-M.P. (peters@imp.univie.ac.at).

LETTERS

Discovery of the progenitor of the type Ia supernova 2007on

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Type Ia supernovae are exploding stars that are used to measure the accelerated expansion of the Universe[1,2] and are responsible for most of the iron ever produced[3]. Although there is general agreement that the exploding star is a white dwarf in a binary system, the exact configuration and trigger of the explosion is unclear[4], which could hamper their use for precision cosmology. Two families of progenitor models have been proposed. In the first, a white dwarf accretes material from a companion until it exceeds the Chandrasekhar mass, collapses and explodes[5,6]. Alternatively, two white dwarfs merge, again causing catastrophic collapse and an explosion[7,8]. It has hitherto been impossible to determine if either model is correct. Here we report the discovery of an object in pre-supernova archival X-ray images at the position of the recent type Ia supernova (2007on) in the elliptical galaxy NGC 1404. Deep optical images (also archival) show no sign of this object. From this we conclude that the X-ray source is the progenitor of the supernova, which favours the accretion model for this supernova, although the host galaxy is older (6–9 Gyr) than the age at which the explosions are predicted in the accreting models.

The two proposed progenitor models of type Ia supernovae are radically different. In the accreting model a prolonged phase of mass transfer precedes the explosion, often identified with the bright so-called supersoft X-ray sources, binary stars in which the mass transfer is believed to be just fast enough to sustain steady nuclear burning on the surface of the white dwarf[9]. In the merger model the mass growth is extremely rapid during the merger, but there is no mass transfer before the explosion, and it is expected that such progenitors will be extremely faint. However, it has been suggested that the mass growth might be slowed down significantly by the rapid rotation of the system[10], easing the problems with the triggering of the explosions when the mass growth is too rapid[11]. In that case there would be thousands of years between the merger and the actual explosion, and the progenitors might also be X-ray sources.

Attempts to distinguish between the models have been based on indirect methods. Calculations of the rate at which supernovae occur as a function of the age of the stellar population show that the mergers occur in populations of all ages whereas the accreting models tend to occur mostly at intermediate ages (~200 Myr to ~2 Gyr)[12,13], and from the statistics of observed supernovae there is a growing evidence for a two-component model for the rates (one component proportional to star formation and one proportional to mass)[14,15]. Other attempts to constrain the progenitors have come from very detailed studies of the spectra of the supernovae[16,17], and attempts have been made to search for the companion stars in supernova remnants[18]. We have taken a different approach by searching for ways to directly detect the progenitor of a type Ia supernova in pre-supernova images of the position in the sky where the supernova occurred.

On 2007 November 5, supernova SN2007on was found in the outskirts of the elliptical galaxy NGC 1404[19]. Optical spectra of the supernova[20] showed that the supernova was of type Ia. The position of the supernova, at RA = 03 h 38 m 50.9 s, dec. = −35° 34′ 30″ (J2000), is about 70″ from the core of the host, corresponding to 8 kpc for a distance of 20 Mpc to NGC 1404[21]. Observations by the SWIFT mission on November 11 detected the supernova in the optical/ultraviolet monitor but not in the X-ray telescope[22]. We analysed the SWIFT data and determined the position of the supernova as RA = 03 h 38 m 50.98 s, dec. = −35° 34′ 31.0″ (J2000), with uncertainty of 1″ (Fig. 1). The highest magnitude is $V \approx 13$ (on November 16), yielding an absolute magnitude of $M_v \approx -18.5$ and making it a rather faint type Ia supernova[23]. NGC 1404 is an elliptical galaxy in the Fornax cluster and its colours suggest that its stellar population is old, with estimates ranging from 6 to 9 Gyr (error about 2 Gyr) and no sign of recent star formation[24,25].

We investigated the supernova position, using archival data before the explosion from the Hubble Space Telescope and Chandra X-ray Observatory, to search for a possible progenitor to the type Ia supernova (Fig. 1). In the Chandra observations obtained in 2003 a source is detected close to the position of the supernova, at the coordinates RA = 03 h 38 m 50.91 s, dec. = −35° 34′ 30.9″ (with a 1σ statistical error of 0.25″, as well as a ~0.5″ error on the absolute astrometric precision, based on correlations between X-ray sources and the images of the region taken by the Two Micron All Sky Survey, 2MASS, and Digitized Sky Survey, DSS). No optical source was detected to an absolute magnitude limit of −4.5. The X-ray source is 0.9 ± 1.3″ (mean ± s.d.) from the supernova, consistent with being its progenitor. The X-ray source is detected with 14.1 ± 4.6 counts, a 4.0σ significance using circular aperture photometry, and 5.0σ based on a wavelet analysis using the program wavdetect. Within a radius of 1′ from the supernova position there are seven detected sources, giving a density of detected sources of 2.2 ± 0.8 per square arcminute. The probability of a chance coincidence of a source within a distance of 1.3″ of the supernova position is therefore 0.3%. Even given the fact that this is not the first trial but the fourth (see below), the likelihood of a chance alignment is very small. As globular clusters are known to be abundant in low-mass X-ray binaries, the alignment of an X-ray source and the supernova would not be so significant if the supernova went off in a globular cluster. But this possibility is excluded by the non-detection of a source in the optical images. We therefore conclude that we have detected the progenitor of 2007on.

With the low number of counts it is impossible to determine the shape of the X-ray spectrum of the progenitor. Instead we investigate the source properties using the number counts in the source and background regions in three different energy bands, S (0.3–1.0 keV), M (1.0–2.0 keV) and H (2.0–8.0 keV). The results are $S = 12$, $M = 4$, $H = 5$, with background expectations of 3.8 ± 2.1,

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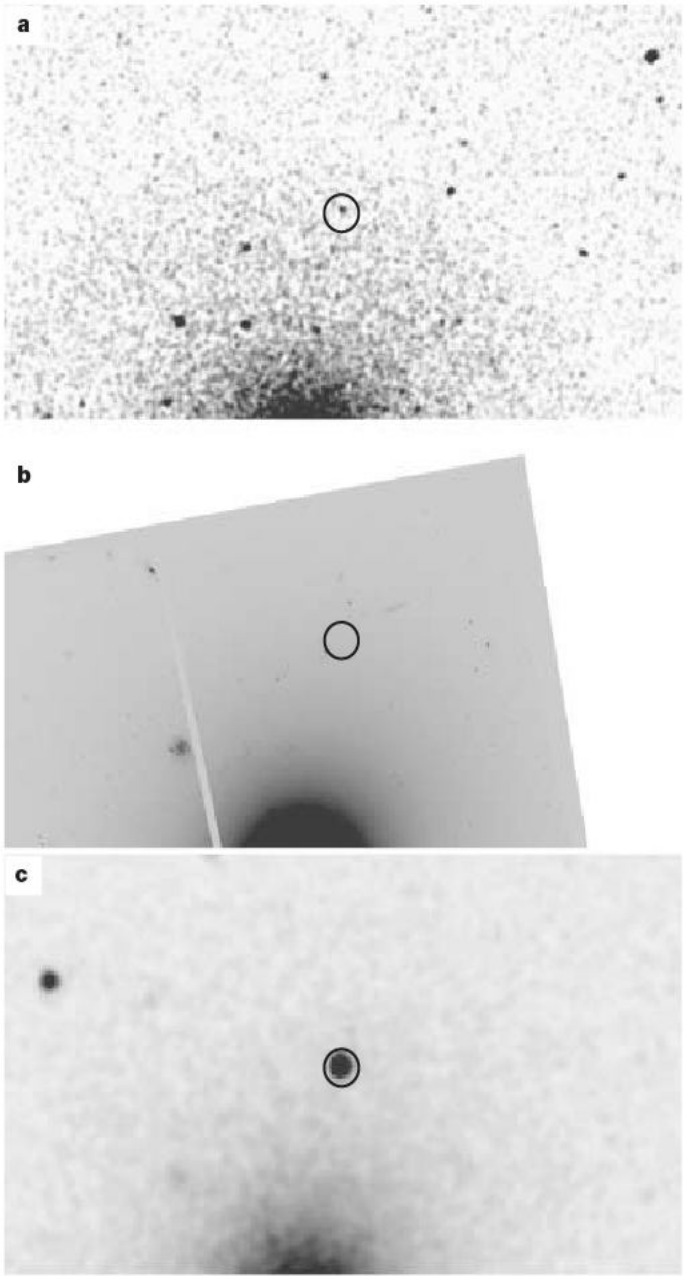

Figure 1 | Images of the region around SN2007on. The images were taken by the Chandra X-ray observatory (**a**, archival pre-supernova image), Hubble Space Telescope (**b**, archival pre-supernova image) and SWIFT (**c**, optical image of supernova), and are smoothed by a Gaussian with a two-pixel FWHM. The circle gives a 5″ radius around the position of the supernova (the circle is much larger than the positional uncertainty). The central parts of NGC 1404 are visible south of the supernova position. There are eight archival Chandra imaging observations with the supernova position within the field of view. Only two of these (Observation ID numbers 2942 and 4174 taken on 2003 February 13 and 2003 May 28, with a combined exposure time of 74.9 ks) have telescope pointings within 6′ of the supernova position and thus enough sensitivity to be useful. We added the two observations, and the resulting image is shown in **a**. Within an aperture of 2″ the X-ray source has 21 counts, whereas a background region in the annulus 2–10″ has 165 counts giving an expectation value of 6.9 counts in the source region. The Poisson probability of having 21 or more counts with this background level is 1.12×10^{-5}, so the source is detected with a significance of 4.0 and the number of source counts is 14.1 ($\pm$4.6). In the Hubble Space Telescope archive there are four images from the Wide Field and Planetary Camera (WFPC2), four from the Advanced Camera for Surveys (ACS) and one from the Near Infrared Camera and Multi-Object Spectrometer (NICMOS) that cover the position of the supernova. No source is present within 2″ of the given position in any of the images. The deepest (ACS) images in the F475W band (760 s, taken 2004 September 10) and F814W band (1.224 ks, taken 2006 August 6) have a limiting magnitude of about 27, corresponding to an absolute magnitude of -4.5. The image with the F475 filter is shown in **b**. In **c** we show a V-band image taken by the SWIFT ultraviolet/optical telescope (2007 November 11).

2.0 ± 1.4 and 1.1 ± 1.2, respectively. Analysis of all (eight) individual images available in the Chandra archive shows no sign of variability. The luminosity of the source for a distance of 20 Mpc is $(3.3 \pm 1.5) \times 10^{37}$ erg s^{-1} (for a flat photon spectrum in each band, a power law with index 2 gives $(2.2 \pm 0.9) \times 10^{37}$ erg s^{-1}).

In the archives there are three more type Ia supernovae in galaxies with a distance of less than 25 Mpc (all lenticular galaxies) and with Chandra observations longer than 10 ks before the supernova explosion. We do not detect an X-ray source at the position of the supernova in any of them. The 3σ upper limits, obtained using the same aperture method as above and assuming the same spectral model, are presented in Table 1, together with the parameters of 2007on.

The discovery of a luminous X-ray source that is likely to be the direct progenitor of 2007on has important consequences for our understanding of type Ia supernovae. The X-ray luminosity is fully consistent with the typical luminosities of supersoft sources[26], which are similar to the expectations from the accreting progenitor model. Also, their absolute magnitudes[26] are around -1 to -2, consistent with the non-detection in the optical images. If in the merger model the explosion immediately follows the actual merger, the progenitor is not expected to emit X-rays before the supernova explosion, and this is therefore inconsistent with the observed progenitor of SN2007on. However, if the lower-mass white dwarf is disrupted at the onset of Roche-lobe overflow and forms a long-lived disk around the more massive white dwarf[10], the merged object may be a strong X-ray source before the explosion. Recent detailed calculations of these objects suggest that they would have X-ray luminosities about an order of magnitude lower than the progenitor that we have discovered[27]. We therefore conclude that our result favours the accreting model. Alternatively, very high accretion rates in the early phases of the evolution of AM CVn systems can also lead to steady burning on white dwarfs, but now of accreted helium[28]. Although the rate of type Ia supernovae from this channel is very low, it is consistent with the properties of the progenitor.

The spectrum of the progenitor is relatively hard, compared with typical supersoft sources which have temperatures below 100 eV. A 100-eV black-body model can be ruled out, but models with 200–300 eV and a modest intrinsic absorption of 10^{21} atoms cm^{-2} are consistent with the observed counts. In addition the age of the stellar population of NGC 1404 could pose a problem for the supersoft source interpretation: the models predict lifetimes only up to about 2 Gyr (refs 11, 12), considerably younger than the inferred age of the population of NGC 1404. This may indicate that the progenitor was a lower-mass

Table 1 | Nearby type Ia supernovae observed with Chandra before the explosion

Supernova	2007on	2006mr	2004W	2002cv
Galaxy	NGC 1404	NGC 1316	NGC 4649	NGC 3190
Galaxy type	Elliptical	Lenticular (Sa)	Lenticular (S0)	Lenticular (Sa)
Distance (Mpc)	20	18.1	15.9	22.4
Observation ID	2942 and 4174	2022	785	2760
Time before supernova	~4 yr	~5 months	~4 yr	~2 months
Count rate (s^{-1})	$(1.9 \pm 0.6) \times 10^{-4}$	$<9.2 \times 10^{-4}$	$<3.2 \times 10^{-4}$	$<3.6 \times 10^{-4}$
Luminosity (erg s^{-1})	$(3.3 \pm 1.5) \times 10^{37}$	$<1.3 \times 10^{38}$	$<3.5 \times 10^{37}$	$<7.9 \times 10^{37}$

system, such as a symbiotic binary, for which hard X-rays have recently been discovered[29]. However, it cannot be excluded that a small population of younger stars is present in the host galaxy of 2007on.

Our discovery opens a new method for the study of type Ia supernova progenitors. This first detection favours the accreting model and the three upper limits on previous supernovae are not strong enough to provide additional constraints. But this does not prove that the other models cannot lead to type Ia supernovae. Searches for supersoft sources in nearby galaxies have resulted in many fewer supersoft sources than expected and needed to explain all type Ia supernovae (see online results at ⟨http://online.itp.ucsb.edu/online/snovae_c07/distefano/⟩), even though the strong variability of these sources complicates the analysis. Also, the growing support for a two-component model for the rate of supernovae may well indicate that the two progenitor models are complementary. Future detections or strong upper limits on pre-supernova X-ray luminosities are important for the understanding of this issue.

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LETTERS

Giant magneto-elastic coupling in multiferroic hexagonal manganites

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The motion of atoms in a solid always responds to cooling or heating in a way that is consistent with the symmetry of the given space group of the solid to which they belong[1,2]. When the atoms move, the electronic structure of the solid changes, leading to different physical properties. Therefore, the determination of where atoms are and what atoms do is a cornerstone of modern solid-state physics. However, experimental observations of atomic displacements measured as a function of temperature are very rare, because those displacements are, in almost all cases, exceedingly small[3–5]. Here we show, using a combination of diffraction techniques, that the hexagonal manganites $RMnO_3$ (where R is a rare-earth element) undergo an isostructural transition with exceptionally large atomic displacements: two orders of magnitude larger than those seen in any other magnetic material, resulting in an unusually strong magneto-elastic coupling. We follow the exact atomic displacements of all the atoms in the unit cell as a function of temperature and find consistency with theoretical predictions based on group theories. We argue that this gigantic magneto-elastic coupling in $RMnO_3$ holds the key to the recently observed magneto-electric phenomenon in this intriguing class of materials[6].

In nature, there are two distinct mechanisms known to induce relatively large atomic displacements. One is a ferroelectric transition of displacive origin involving a so-called soft mode, in which certain atoms move below a Curie temperature in such a way that the solid loses its inversion symmetry and becomes ferroelectric with non-centro symmetry[3]. This happens in the perovskite $BaTiO_3$, for which the atomic displacement of Ti atoms is the main driving force behind its ferroelectric transition at 403 K (ref. 4). Another mechanism for relatively big atomic displacements is found in systems in which the ground-state degeneracy is lifted up by some kind of structural distortion[5]. A good example is perovskite transition metal oxides having Mn^{3+} ions with a d^4 configuration, in which the twofold degeneracy of the e_g levels splits, leading to diverse physical properties, such as the well-known colossal magneto-resistance observed in manganites[7].

Atomic displacements arising from either of the two mechanisms with symmetry-lowering transitions can be as large as a few per cent of their lattice constants: for example, the atomic displacement seen in the ferroelectric $BaTiO_3$ is in the range 0.05–0.4 Å (ref. 4). Apart from these two exceptional cases, atomic displacements reported for other numerous ordinary materials are mostly extremely small, often of the order of 10^{-5} Å. Therefore, detailed studies of how the atoms of a given solid move as a function of temperature are almost non-existent, in particular when the solid under investigation undergoes an isostructural transition without breaking its high-temperature symmetry.

For rare-earth elements with relatively smaller ionic radius, $RMnO_3$ forms hexagonal manganites, whereas for rare-earth elements having larger ionic radius $RMnO_3$ forms an orthorhombic structure[8,9]. Despite having the same chemical formula, there is a very important difference between the two structures. As shown in Fig. 1a, the MnO_5 bipyramids of the hexagonal structure form a layered structure on the *a*–*b* plane and, at the same time, they are well-separated from one another along the *c* axis by the rare-earth-element layers, leading to a natural two-dimensional network of the magnetic Mn atoms. Because of the disparate chemical environment surrounding the Mn atoms—unlike the Mn ion of the MnO_6 octahedron with a t_{2g}–e_g splitting—the Mn ions of the MnO_5 bipyramid have two low-lying doublets (xz, yz) and (xy, x^2-y^2) and one singlet state $3z^2-r^2$, in the order of increasing energies[10]. Therefore, the four *d* electrons of the Mn^{3+} of the hexagonal manganites occupy the two low-lying doublets and so there is no orbital degeneracy left, unlike in its counterpart in the MnO_6 octahedron of the orthorhombic manganites[11]. This structural difference makes the physics of the hexagonal manganite markedly different from that of the orthorhombic one.

The hexagonal manganites undergo a paraelectric–ferroelectric transition at high temperatures[12] and, simultaneously, the crystal structure changes from $P6_3/mmc$ to $P6_3cm$. In the ferroelectric phase of the $P6_3cm$ space group, Mn is at $x \cong 1/3$, forming a nearly ideal triangular lattice of Mn ions, as shown in Fig. 1b. Each Mn ion is connected to another either through one O3 atom or one of two O4 atoms located on the same *a*–*b* plane (see Fig. 1b). Because of the intrinsically frustrated nature of the triangular lattice with antiferromagnetic interaction, Mn $S=2$ moments cannot order until well below their Curie–Weiss temperatures; these are $\theta_{CW}=-500$ K and the Neels temperature $T_N=75$ K for $YMnO_3$ (see the inset of Supplementary Fig. 1b). Therefore, the triangular lattice of the Mn atoms exhibits strong geometrical frustration effects with a so-called frustration parameter, $f=|\theta_{CW}|/T_N$, which is as large as 6.7 for $YMnO_3$. When they eventually order, a clear anomaly is observed in the magnetic susceptibility (Supplementary Fig. 1), the heat capacity (Supplementary Fig. 2) and the neutron-scattering data. For example, apart from the large frustration number *f*, the heat capacity shows that almost a third of the total magnetic entropy is released above T_N (see Supplementary Fig. 2), while there is strong magnetic diffuse scattering with strong temperature dependence in neutron diffraction data in the supposedly paramagnetic phase[13]. Inelastic neutron scattering studies[13,14] also found unusually strong spin fluctuations still persisting even well below T_N.

Pioneering work in the 1960s[15] established that these hexagonal manganites have ferroelectric as well as antiferromagnetic transitions within a single compound. Recently there has been renewed interest

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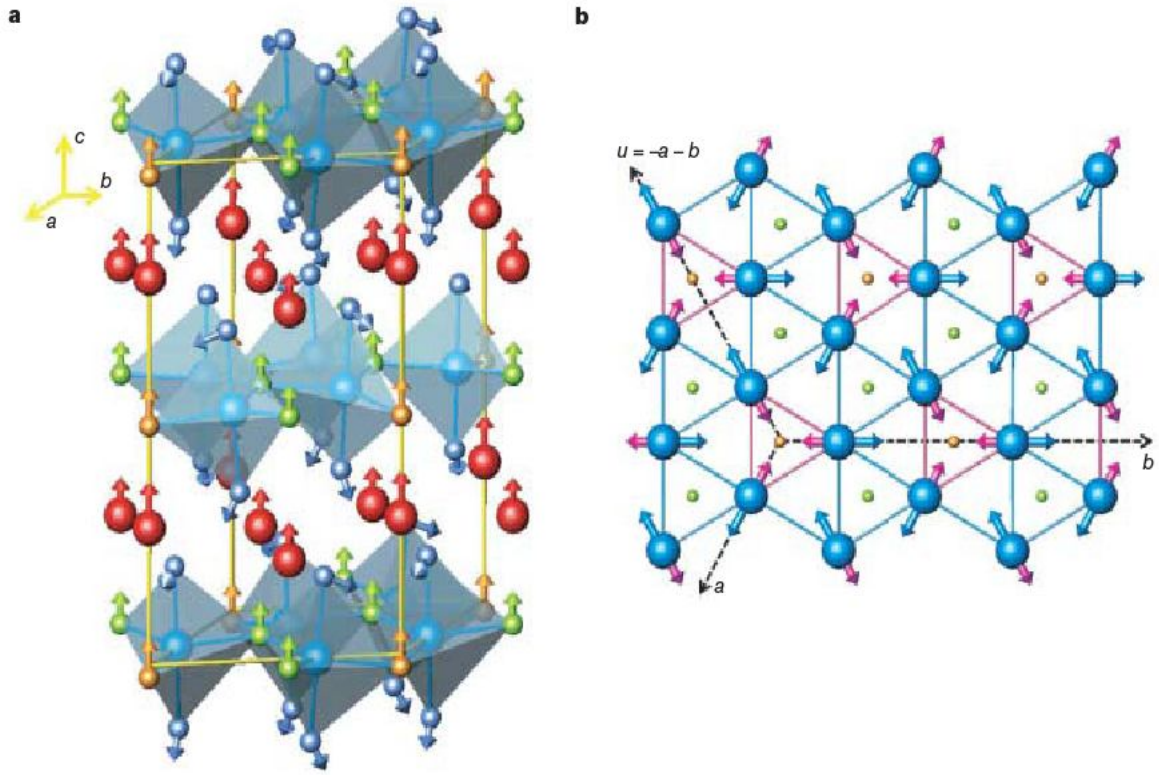
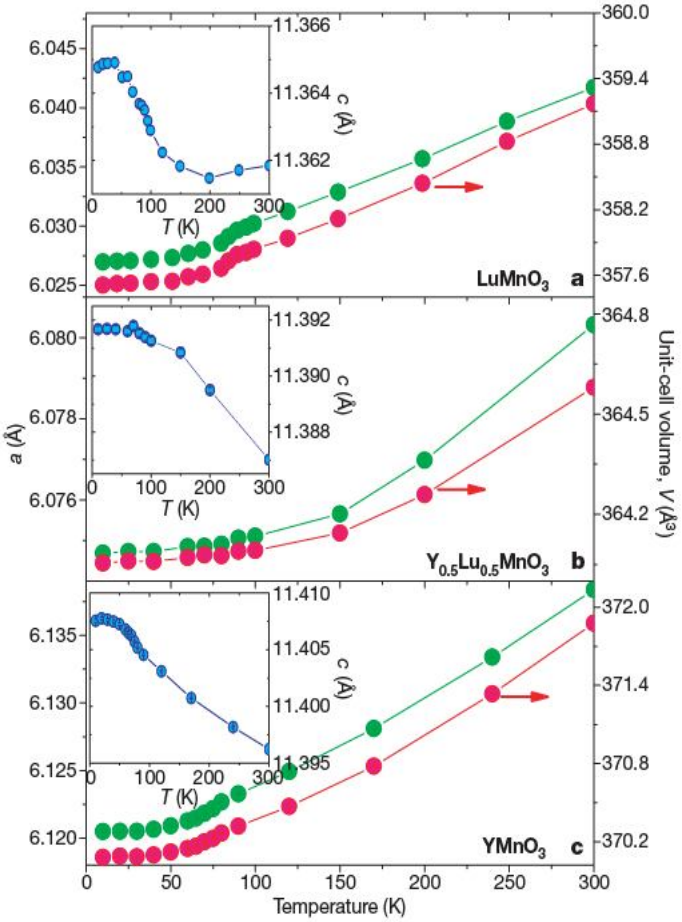


Figure 1 | Crystal structure of hexagonal $RMnO_3$ with arrows indicating experimentally observed atomic displacements. **a**, Unit cell of the hexagonal structure: Y atoms (large red circles), O1 and O2 atoms (small blue circles) and all the other atoms as in **b**, with all the arrows representing the direction of atomic displacements. **b**, Model showing how Mn atoms (large blue circles) move below T_N with respect to O3 (small orange circles) for $YMnO_3$ (blue arrows) and $LuMnO_3$ (purple arrows). The small green circles represent O4.



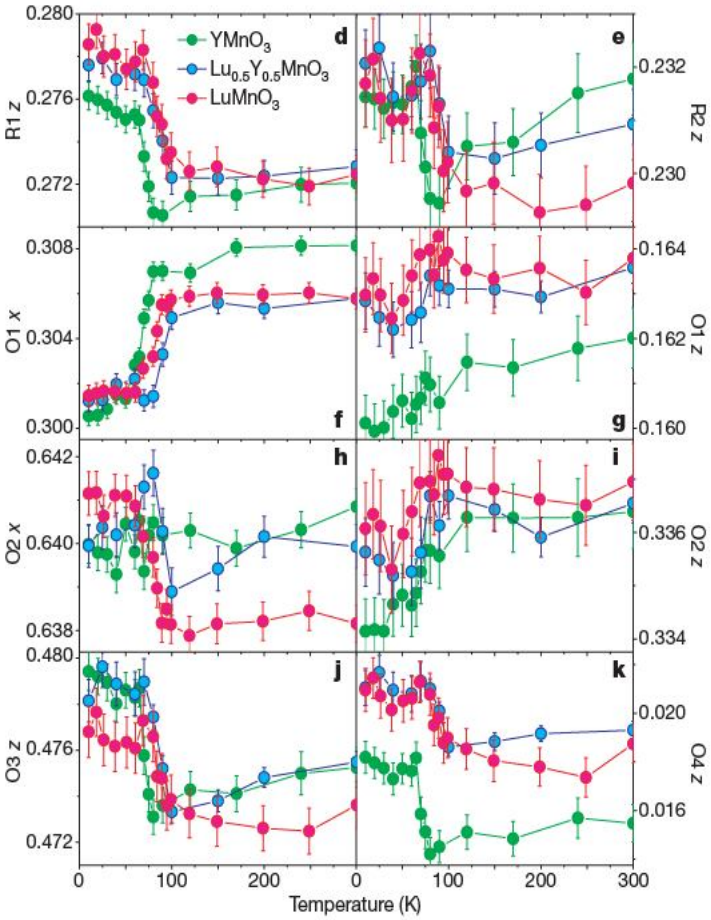


Figure 2 | Temperature dependence of the lattice constants and the unit-cell volume together with atomic positions. **a–c**, Temperature dependences of the lattice constants a and c and the unit-cell volume V obtained from Rietveld refinement of neutron powder diffraction data taken using the SIRIUS beamline at the KEK. **d–k**, Temperature dependence of the atomic positions of all the atoms, except for Mn ions, at six crystallographically different positions. (For example, 'R1 z' is the z value of the R1 atom.) Error bars are the standard deviation obtained from the Rietveld refinement using RIETAN-2001T.

in the 'multiferroic' behaviour of these hexagonal manganites. Early hints of a long-sought coupling between the ferroelectric and antiferromagnetic order parameters can be found in the anomaly of the dielectric constant at T_N and the strikingly similar domain structures of both origins[16,17]. The magnetic phase of hexagonal $HoMnO_3$ can be controlled by external electric fields, which is the realization of the magneto-electric effect or the multiferroic phenomenon[6]. Despite mounting and unquestioned experimental evidence for magneto-electric effects in the hexagonal manganites, how such coupling between the two disparate order parameters actually occurs at a microscopic level still remains unknown.

When the hexagonal manganites undergo antiferromagnetic transitions, there are also distinct anomalies in the structure and thermal conductivity[18–21]. For example, both the *a* axis lattice constant and the unit-cell volume exhibit a sudden drop at T_N, while the *c* axis expands at the same temperature (see Fig. 2a–c). According to our structural results (see Supplementary Figs 3, 4 and 5), no symmetry change occurs at all in the temperature range of interest from 300 to 3 K. We analysed high-resolution neutron diffraction data taken on three samples—$YMnO_3$, $(Y_{0.5}Lu_{0.5})MnO_3$ and $LuMnO_3$—from 300 to 10 K, using the space group $P6_3cm$. Representative data are shown in Supplementary Fig. 5 and the summary of our Rietveld refinement results is given in Supplementary Table 1.

From this detailed analysis a striking structural anomaly emerges. As shown in Fig. 2d–k, all seven atoms belonging to the same unit cell exhibit significantly large atomic displacements at T_N: the arrows in Fig. 1 indicate the experimentally observed directions of the atomic displacements. We note that the atomic displacement found at T_N is as large as approximately 0.05–0.09 Å, which is almost comparable to those reported for archetypal ferroelectric materials. We stress that for the hexagonal manganites there are no orbital degrees of freedom, which could otherwise induce similarly large atomic displacements for compounds having Mn^{3+} ions, through the familiar Jahn–Teller mechanism[5]. Also, our hexagonal manganites undergo an isostructural transition well below their ferroelectric transition points.

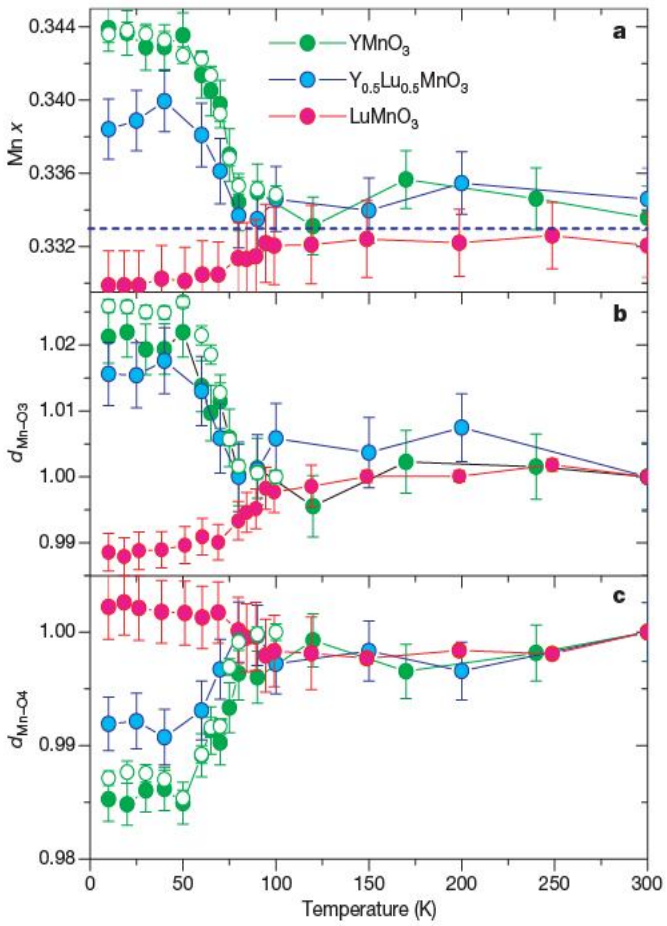


Figure 3 | Temperature dependence of the Mn *x* position and the Mn–O bond distances obtained from high-resolution neutron and synchrotron powder diffraction experiments. **a**, Mn *x* positions. The dashed line indicates a Mn position ($x = 1/3$) for an ideal 2D triangular network consisting of Mn moments. Mn–O3 (**b**) and Mn–O4 (**c**) bond distances *d* are normalized with respect to the value at 300 K. Open symbols represent data points for $YMnO_3$ that were obtained from the analysis of synchrotron powder diffraction experiments at the SLS. Error bars are the standard deviation obtained from the Rietveld refinement using RIETAN-2001T and Fullprof.

This unprecedented large atomic displacement seen for all the atoms of the hexagonal $RMnO_3$ provides an opportunity for us to check the long-held, yet untested, theory of isostructural transitions. By comparing the experimentally observed atomic movements with theoretical predictions based on group theories, we found that all our experimental atomic displacements are indeed consistent with the τ_1 mode of the group theories. (See Supplementary Fig. 6 and Supplementary Table 2.)

What drives the gigantic magnetic-elastic coupling observed for the hexagonal manganites? We believe that the answer lies in the atomic displacement of the Mn atoms. Like the Y, Lu and O atoms shown in Fig. 2d–k, the Mn atoms also exhibit marked changes in their positions below T_N. As already noted, Mn is positioned at $x \cong 1/3$ in the paramagnetic phase, which makes an almost ideal triangular arrangement of the Mn atoms in the high-temperature phase. However, as soon as the Mn moments start ordering below T_N, their position shifts drastically away from the ideal value of $x = 1/3$. For example, the *x* value increases from 0.3330(17) at 300 K to 0.3423(13) at 10 K for $YMnO_3$. A slightly smaller change is observed for $LuMnO_3$, but with an opposite sign. The temperature dependence of $(Y_{0.5}Lu_{0.5})MnO_3$ is just between those of $YMnO_3$ and $LuMnO_3$ (see Fig. 3a). The overall observed relative shift in the Mn *x* position amounts to almost 3.3%, which is comparable to the atomic displacement of Ti atoms in $BaTiO_3$ (ref. 4). This abnormally large shift in the Mn position is consistent with an anomaly seen at T_N in the optical data of $LuMnO_3$ (ref. 22). We note that this large atomic displacement produces a further coupling to electric dipole moments[19]. Using a model described elsewhere[19], we have found that the induced electric dipole moment *P* is proportional to the square of the magnetic moments, as expected in the absence of orbital degrees of freedom (see Supplementary Fig. 7) Therefore, the magnetic-elastic coupling is the primary source of the magneto-electric coupling (that is, the multiferroic phenomenon) of this intriguing class of materials.

Because of the distortion in the triangular lattice of the Mn moments, the equal distance of Mn–O bonds on the *a*–*b* plane now splits into two different bonds below T_N: Mn–O3 and Mn–O4 (see Fig. 3b, c). This splitting naturally leads to two different magnetic exchange interactions J_1 and J_2 on the *a*–*b* plane, which are magnetically much stronger than along the *c* axis[14]. According to the inelastic neutron scattering experiments performed on $YMnO_3$, the two exchange integrals on the *a*–*b* plane differ by as much as 70%: $J_1 = 3.4$ meV and $J_2 = 2.0$ meV. A similarly large difference in the two exchange integrals was also found in our recent inelastic neutron-scattering studies of $LuMnO_3$. The strong magnetic frustration effects seen in both bulk data and neutron-scattering results mean that our observations could also be due to some kind of geometrical frustration effect. A geometrically frustrated system such as the triangular lattice of the Mn moments can sometimes reduce its huge thermodynamic degeneracy[23]. However, we note that any simple model based on the geometrical frustration effects alone appears to be at variance with the current understanding of the magnetic structure and, in particular, the opposite displacements of the Mn atoms seen for $YMnO_3$ and $LuMnO_3$. Another possible scenario is the probable role of some small, pre-existing off-centring of the Mn ion. Although it seems to be weak in the paramagnetic phase, it can be amplified below T_N through the unusually strong magneto-elastic coupling.

What is novel about our results is that the hexagonal $RMnO_3$ undergoes such a strong change without breaking the high-temperature symmetry. Instead, it gives rise to giant atomic displacements for every atom within the unit cell. Through this unusual behaviour of atomic movements, it produces the desired coupling between the ferroelectric moment and the antiferromagnetic moment, that is, a magneto-electric coupling. Our study shows how such magneto-electric coupling occurs at an atomic level and opens up a new door to this currently very active field of multiferroic systems[24,25].

In summary, we have found that the hexagonal manganites undergo an isostructural transition at T_N, simultaneously producing giant atomic displacements for every atom in the unit cell. Surprisingly, this happens without either soft-mode degrees of freedom (unlike the usual ferroelectric materials) or orbital degrees of freedom (unlike orthorhombic manganites). This extremely large magneto-elastic coupling, larger by two orders of magnitude than in any magnetic materials, is the primary origin of their multiferroic phenomenon.

METHODS SUMMARY

Sample preparation and characterization of bulk properties. We prepared all our samples of $(Y_{1-x}Lu_x)MnO_3$ (where $x = 0$, 0.5 and 1), using starting materials of Y_2O_3, Lu_2O_3 and Mn_2O_3 of 99.999% with the solid-state reaction method. The initial sintering was done at 1,000 °C for 48 h, followed by the final heat treatment at 1,300 °C for 24 h. Before subsequent measurements, all our samples were subjected to X-ray diffraction measurements (D/MaX-2200 Ultima). For our single-crystal X-ray diffraction experiment at low temperatures, we grew single-crystal $YMnO_3$ using a commercial infrared mirror furnace (Crystal Systems, Japan).

High-resolution neutron/synchrotron/X-ray diffraction experiments. Our powder neutron diffraction experiments were performed using the SIRIUS beamline at KEK, Japan, which has a resolution of $\Delta l/l = 9 \times 10^{-4}$. To investigate the crystal structure of $YMnO_3$ below T_N, we performed high-resolution synchrotron powder diffraction experiments using two beamlines: the 8C2 beamline at the Pohang Accelerator Laboratory, Korea, and the Materials Science beamline at the Swiss Light Source (SLS), Switzerland. Both beamlines have similar reported resolution values of $\Delta l/l = 5 \times 10^{-5}$ (ref. 26). All our data were analysed using Rietveld refinement programmes: RIETAN-2001T and Fullprof[27,28]. As a further check of the structure of $YMnO_3$ below T_N, we carried out high-resolution X-ray single-crystal diffraction at 3 K using a home-made set-up at Tohoku University.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions J.-G.P. planned the study and supervised the analysis. S.L. synthesized all powder samples and carried out powder diffraction experiments with the help of M.K., M.Y., T.K., F.G. and N.S. S.L. analysed the diffraction data together with A.P. and T.K. K.-H.J. measured and analysed the susceptibility and heat capacity data with J.-G.P. S.-W.C. provided single-crystal $YMnO_3$. J.-G.P. performed the single-crystal X-ray experiments with the help of H.K. and Y.N. J.-G.P. discussed the results with S.L., A.P., T.K., S.-W.C. and Y.N., and wrote the manuscript.

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LETTERS

Microfibre–nanowire hybrid structure for energy scavenging

Yong Qin[1]*, Xudong Wang[1]* & Zhong Lin Wang[1]

A self-powering nanosystem that harvests its operating energy from the environment is an attractive proposition for sensing, personal electronics and defence technologies[1]. This is in principle feasible for nanodevices owing to their extremely low power consumption[2–5]. Solar, thermal and mechanical (wind, friction, body movement) energies are common and may be scavenged from the environment, but the type of energy source to be chosen has to be decided on the basis of specific applications. Military sensing/surveillance node placement, for example, may involve difficult-to-reach locations, may need to be hidden, and may be in environments that are dusty, rainy, dark and/or in deep forest. In a moving vehicle or aeroplane, harvesting energy from a rotating tyre or wind blowing on the body is a possible choice to power wireless devices implanted in the surface of the vehicle. Nanowire nanogenerators built on hard substrates were demonstrated for harvesting local mechanical energy produced by high-frequency ultrasonic waves[6,7]. To harvest the energy from vibration or disturbance originating from footsteps, heartbeats, ambient noise and air flow, it is important to explore innovative technologies that work at low frequencies (such as <10 Hz) and that are based on flexible soft materials. Here we present a simple, low-cost approach that converts low-frequency vibration/friction energy into electricity using piezoelectric zinc oxide nanowires grown radially around textile fibres. By entangling two fibres and brushing the nanowires rooted on them with respect to each other, mechanical energy is converted into electricity owing to a coupled piezoelectric–semiconductor process[8,9]. This work establishes a methodology for scavenging light-wind energy and body-movement energy using fabrics.

The fibres used in our experiments were Kevlar 129 fibres, which have high strength, modulus, toughness and thermal stability. ZnO nanowires were then grown radially on the fibre surface using a hydrothermal approach[10]. A typical scanning electron microscopy (SEM) image of a Kevlar fibre covered by ZnO nanowires is shown in Fig. 1a. Along the entire length of the fibre, ZnO nanowires grew radially and exhibited a very uniform coverage and well preserved cylindrical shape. Some splits in the nanowire arrays can be identified (Fig. 1b), which were produced owing to the growth-induced surface tension in the seeding layer. All of the ZnO nanowires are single crystalline, and have a hexagonal cross-section with a diameter in the range ~50–200 nm and a typical length of ~3.5 μm. Their top and side surfaces are smooth and clean, indicating that they are able to form the reliable metal–semiconductor junctions needed for the nanowire nanogenerators. The space between the nanowires is of the order of a few hundred nanometres, which is large enough for them to be bent to generate the piezoelectric potential[11]. The nanowires' tips are separated from each other owing to their small tilting angles ($<\pm 10°$), but their bottom ends are tightly connected (Fig. 1b inset). As a result, a continuous ZnO film at the nanowires' roots served as a common electrode for signal output. Previous experiments showed that AFM (atomic force microscope) manipulation of a solution-grown ZnO nanowire can give up to 45 mV output voltage[12].

To maintain the high flexibility of the fibre after growing a crystalline film and nanowires, a surface coating strategy was introduced to improve the mechanical performance of the fibre and the binding of nanowires. Two layers of tetraethoxysilane (TEOS) were infiltrated—one above and one below the ZnO seed layer—as binding agents (Fig. 1c). The Si–O bonds in TEOS are highly reactive with the OH^- groups on the ZnO surface[13], and its organic chains firmly bind to the body of the aromatic polyamide fibre. As a result, the ZnO seed layer and the fibre core were tightly bound with each other by a thin layer of TEOS. Furthermore, since TEOS could easily form cross-linked chains, the nanowires were firmly bundled and bound together at their roots and fixed on the ZnO seeding layer,

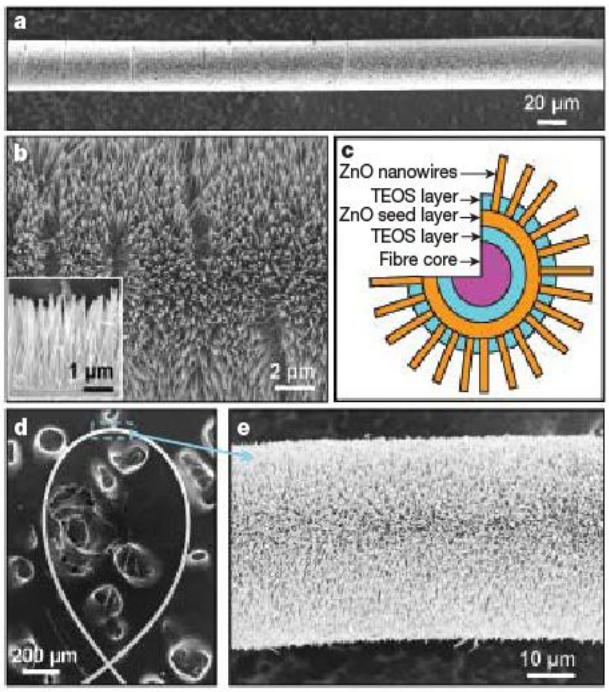

Figure 1 | Kevlar fibres coated with ZnO nanowires. **a**, An SEM image of a Kevlar fibre covered with ZnO nanowire arrays along the radial direction. **b**, Higher magnification SEM image and a cross-section image (inset) of the fibre, showing the distribution of nanowires. **c**, Diagram showing the cross-sectional structure of the TEOS-enhanced fibre, designed for improved mechanical performance. **d**, SEM image of a looped fibre, showing the flexibility and strong binding of the nanowire layer. **e**, Enlarged section of the looped fibre, showing the distribution of the ZnO nanowires at the bending area.

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successfully preventing them from scratching/stripping off during mechanical brushing/sliding. Even when the fibre was looped into a circle of ~1 mm in diameter, no cracks, loose pieces or peel-offs were observed in the ZnO nanowire coating layer (Fig. 1d). At the bending area, the radially aligned morphology of the ZnO nanowires was also very well preserved (Fig. 1e), clearly demonstrating its toughness under mechanical deformation and bending.

In order to demonstrate the power generation ability of the ZnO-nanowire-covered fibres, a double-fibre model system was designed (Fig. 2a). Two fibres, one coated with a 300-nm-thick gold layer and the other as-grown, were entangled to form the core for power generation. The relative brushing between the two fibres was simulated by pulling/releasing the string using an external rotor with a controlled frequency. The gold-coated fibre was connected to the external circuit as the nanogenerator's output cathode. The pulling force ensured a good contact between the two fibres, as shown by an optical microscopy image (Fig. 2b).

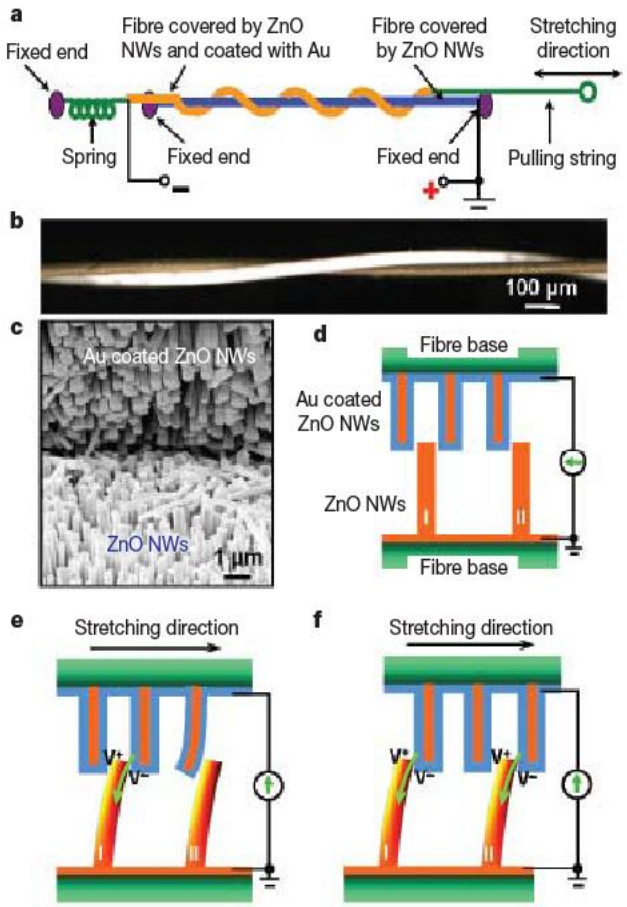

Figure 2 | Design and electricity-generating mechanism of the fibre-based nanogenerator driven by a low-frequency, external pulling force. **a**, Schematic experimental set-up of the fibre-based nanogenerator. **b**, An optical micrograph of a pair of entangled fibres, one of which is coated with Au (in darker contrast). **c**, SEM image at the 'teeth-to-teeth' interface of two fibres covered by nanowires (NWs), with the top one coated with Au. The Au-coated nanowires at the top serve as the conductive 'tips' that deflect/bend the nanowires at the bottom. **d**, Schematic illustration of the teeth-to-teeth contact between the two fibres covered by nanowires. **e**, The piezoelectric potential created across nanowires I and II under the pulling of the top fibre by an external force. The side with positive piezoelectric potential does not allow the flow of current owing to the existence of a reverse-biased Schottky barrier. Once the nanowire is pushed to bend far enough to reach the other Au-coated nanowire, electrons in the external circuit will be driven to flow through the uncoated nanowire due to the forward-biased Schottky barrier at the interface. **f**, When the top fibre is further pulled, the Au-coated nanowires may scrub across the uncoated nanowires. Once the two types of nanowires are in final contact, at the last moment, the interface is a forward biased Schottky, resulting in further output of electric current, as indicated by arrowheads. The output current is the sum of all the contributions from all of the nanowires, while the output voltage is determined by one nanowire.

In this design, the gold-coated ZnO nanowires acted as an array of scanning metal tips that deflected the ZnO nanowires rooted at the other fibre; a coupled piezoelectric and semiconducting property resulted in a process of charge creation and accumulation and charge release[8,9]. The gold coating completely covered the ZnO nanowires and formed a continuous layer along the entire fibre. A successful coating was confirmed by its metallic *I*–*V* characteristic (Supplementary Fig. 1). Once the two fibres were firmly entangled together, some of the gold-coated nanowires penetrated slightly into the spaces between the uncoated nanowires rooted at the other fibre, as shown by the interface image in Fig. 2c. Thus, when there was a relative sliding/deflection between them, the bending of the uncoated ZnO nanowires produced a piezoelectric potential across their width, and the Au-coated nanowires acted as the 'zigzag' electrode (as for the DC nanogenerator[6]) for collecting and transporting the charges.

Figure 2d–f illustrates the charge generation mechanism of the fibre nanogenerator. Like the deflection of a nanowire by an AFM tip[8], when the top fibre move to the right, for example, the gold-coated nanowires bend the uncoated ZnO nanowires to right (for simplicity of description, we assume that the gold-coated nanowires are much stiffer and suffer little bending). Piezoelectric potential is thus generated across the uncoated nanowire owing to its piezoelectric property, with the stretched surface positive (V^+) and the compressed surface negative (V^-)[8]. The positive-potential side has a reverse-biased Schottky contact with the gold (see the *I*–*V* curve in Supplementary Fig. 2) that prevents the flow of current, while the negative-potential side has a forward-biased Schottky contact with the gold that allows the current to flow from the gold to the nanowire. As the density of the nanowires is high (Fig. 1b), it is very likely that a bent nanowire rooted at the uncoated fibre touches the backside of another gold-coated nanowire after subjecting to bending (such as nanowire I in Fig. 2e). In this case, the negative-potential surface of the ZnO nanowire contacts the gold layer, so the Schottky barrier at the interface[14] is forward biased, resulting in a current flowing from the gold layer into the ZnO nanowire. Then, when the top fibre keeps moving further towards the right (Fig. 2f), the gold-coated nanowires scan across the ZnO nanowires' tips and reach their negatively charged sides (nanowires I and II in Fig. 2f). Therefore, more current will be released through the forward-biased Schottky barriers (Fig. 2f). This means that the currents from all of the nanowires will add up constructively regardless of their deflection directions, even in the same cycle of pulling.

The output voltage is defined by the characteristic of one nanowire, and the sign of the voltage does not change in response to the deflection configuration of the nanowire owing to the rectifying effect of the Schottky barrier at the Au–ZnO interface. The same effect is expected if the top fibre is driven to the left. Owing to the similar mechanical properties of the top and bottom nanowires, the gold-coated nanowires could also be possibly bent by the nanowires rooted at the uncoated fibre, but this does not affect the mechanism presented in Fig. 2. For the gold-coated fibre, all of the nanowires are completely covered by a thick gold layer, and they can be considered as an equal-potential electrode (Supplementary Fig. 1) connected to the external measurement circuit. Thus, the role played by these ZnO nanowires was only to act as a template for supporting the Au coating, and no piezoelectric charges will be preserved inside the gold-coated nanowires.

The short-circuit current (I_{sc}) and open-circuit voltage (V_{oc}) were measured to characterize the performance of the fibre nanogenerators. The pulling and releasing of the gold-coated fibre was accomplished by a motor at a controlled frequency. The resulting current signals detected at 80 r.p.m. (revolutions per minute; 60 r.p.m. = 1 Hz) are shown in Fig. 3a. A 'switching polarity' testing method was applied during the entire measurement to rule out system artefacts. When the current meter was forward-connected to the

nanogenerator, which means that the positive and negative probes were connected to the positive and negative electrodes of the nanogenerator as defined in Fig. 2a, respectively, ~5 pA positive current pulses were detected at each pulling-releasing cycle (blue curve in Fig. 3a). Negative current pulses with the same amplitude were received (pink curve in Fig. 3a) when the current meter was reverse-connected, with its positive and negative probes connected to the negative and positive electrodes of the nanogenerator, respectively. The small output current (~5 pA) is attributed mainly to the large loss in the fibre due to an extremely large inner resistance ($R_i \approx 250$ MΩ; see Supplementary Fig. 3). This large inner resistance of the fibre-based nanogenerator is probably due to cracks in the ZnO seed layer directly adjacent to the fibre that are caused by the structural incompatibility and large difference in thermal expansion coefficients. Reducing R_i of the fibre nanogenerator is an effective way to improve its power output efficiency, which is demonstrated in Fig. 4c.

The inverted output current signals confirmed that the current was indeed originated from the fibre nanogenerator. The shapes of the positive and negative current pulse are enlarged and shown in Fig. 3c and d, respectively, from which a double-peak feature is clearly observed for each cycle of pulling the fibre.

Open-circuit voltage was also measured by the switching polarity method. Corresponding positive and negative voltage signals were received when the voltage meter was forward- and reverse-connected to the fibre nanogenerator, respectively (Fig. 3b). The amplitude of the voltage signal in this case was ~1–3 mV (see also Supplementary Fig. 4). As for the current signal, a double-peak feature was also observed in both positive and negative voltage signals (Fig. 3e and f).

The double peaks in the output signal (Fig. 3c–f) are related to the cycle motion of the fibre. When the fibre was pulled to the right-hand-side during stretching and then retracted back by the spring

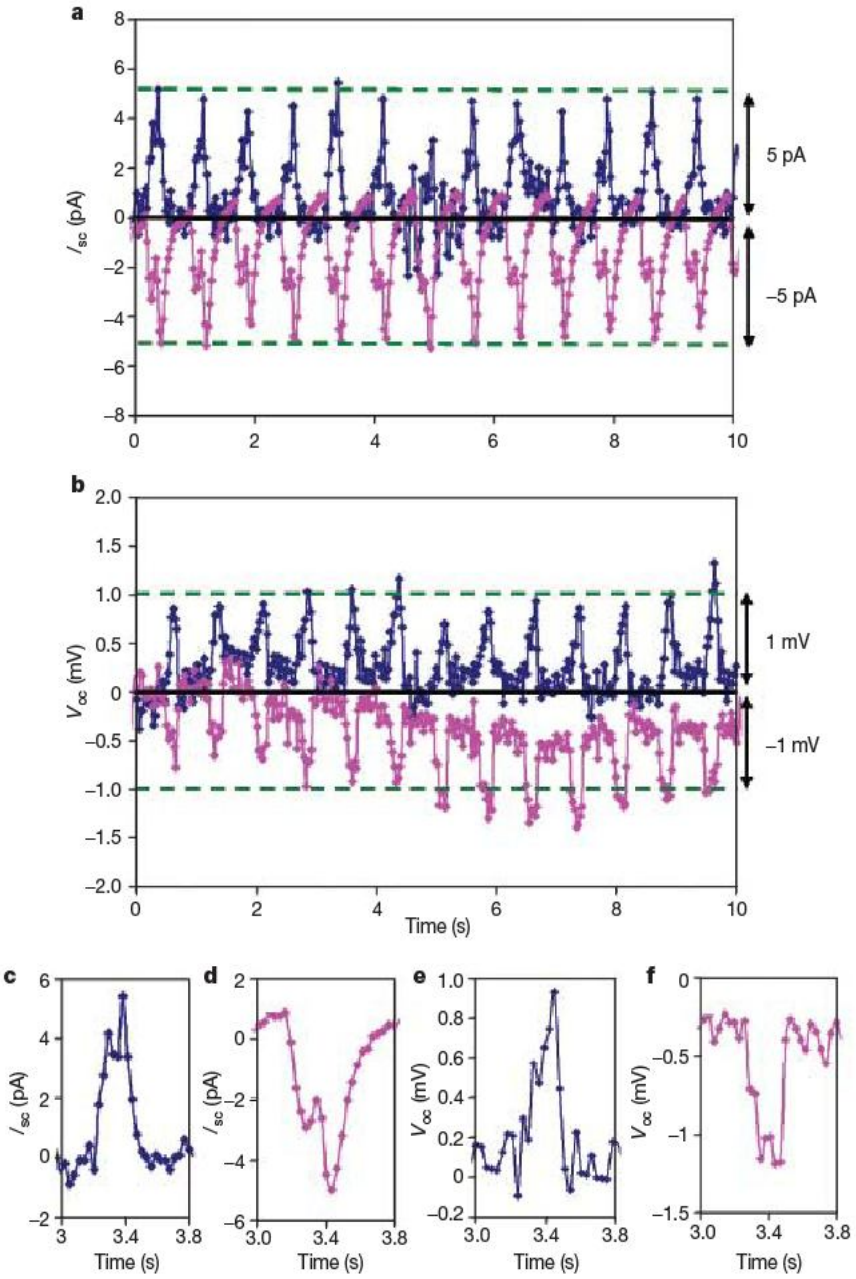

Figure 3 | Electric output of a double-fibre nanogenerator. **a**, The short-circuit output current (I_{sc}) and **b**, the open-circuit output voltage (V_{oc}) of a double-fibre nanogenerator measured by applying an external pulling force at a motor speed of 80 r.p.m. When the measurement circuit was forward-connected to the nanogenerator, which means that the positive and negative probes were connected to the positive and negative output electrodes of the nanogenerator, respectively, the output signals are represented by a pink curve. By reversing the polarity of the connection of the nanogenerator output electrodes, the output signals are presented by the blue curve. **c**, **d**, Magnified output current and **e**, **f**, output voltage for a single cycle of the fibre pulling. The background introduced by the measurement circuit was removed in the displayed plots.

when released, the mechanism illustrated in Fig. 2 remained the same. Although the two moving directions were opposite, the currents generated flowed in the same direction, and a delay of ~0.2 s of one with respect to the other produced double peaks in the resultant output current and voltage. This is a general phenomenon that existed in every output pulse regardless of its polarity. A slight difference in the magnitude of the double peaks may be related to the speed at which the fibre was pulled and retracted.

To exclude the possible contribution made by friction-induced electrostatic charges during the relative sliding/brushing of the two fibres, we have used two fibres both with or without Au coating. For the fibres both without Au coating, no output electric signal was detected (Supplementary Fig. 5a): and for the fibres both with Au coating, no output electricity was detected either (Supplementary Fig. 5b). This means that the observed signal is unrelated to friction induced charging/discharging.

The fibre nanogenerator also exhibited good performance over a range of frequencies. Short-circuit current was measured while the speed of the driving motor was varied from 80 to 240 r.p.m. (Supplementary Fig. 6). The current density decreased almost linearly with the increase of frequency (Supplementary Fig. 7). Owing to the relatively high friction force between the entangled fibres, it takes time for the fibre to recover to its original shape after releasing. As the frequency increases, the time intervals left for the spring to retract the fibre became shorter and shorter, thus, the fibre was not fully recovered to its static shape before the next pulling started, resulting in less travelling distance for the fibre and the smaller electric output.

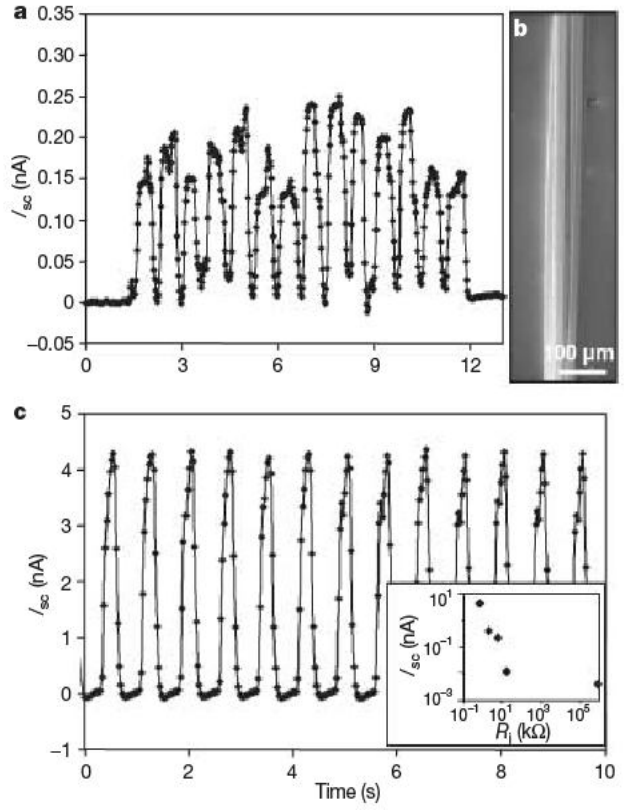

Figure 4 | Output improvements. **a**, The short-circuit output current (I_{sc}) of the multi-fibre nanogenerator operated at 80 r.p.m. A broadening in the output current peaks is observed due to the unsynchronized movement among the fibres. **b**, SEM image of the multi-fibre nanogenerator composed of six ZnO-nanowire-coated fibres, three of which were gold-coated. This shows the possibility of using a bundle of nanowires as a yarn for enhancing the output current. **c**, Enhancement of output current by reducing the inner resistance (R_i) of the nanogenerator. By coating the surface of the fibre first with a conductive layer, then depositing ZnO seeds, the inner resistance of the nanogenerator was reduced by orders of magnitude. The background introduced by the measurement circuit was removed in the displayed plots.

After demonstrating the electricity generation principle, a few approaches were investigated to increase the output power and prototype integration. To simulate a practical fabric made of yarn, a single yarn made of 6 fibres was tested; three fibres were covered with nanowires and coated with Au, and three were only covered with nanowires. All of the gold-coated fibres were movable in the testing (Fig. 4b). At a motor speed of 80 r.p.m., an average current of ~0.2 nA was achieved (Fig. 4a), which is ~30–50 times larger than the output signal from a single-fibre nanogenerator: this is due to the substantially increased surface contact area among the fibres. The width of each pulse was broadened, apparently due to the unsynchronized movement and a relative delay in outputting current among the fibres.

Reducing the inner resistance of the fibre and the nanowires was found to be effective for enhancing the output current. By depositing a conductive layer directly onto the fibre before depositing the ZnO seeds, the inner resistance of the nanogenerator was reduced from ~1 GΩ to ~1 kΩ; thus the output current I_{sc} of a double-fibre nanogenerator was increased from ~4 pA to ~4 nA (Fig. 4c). The current I_{sc} is approximately inversely proportional to the inner resistance of the nanogenerator (inset in Fig. 4c). This study shows an effective approach for increasing the output current. In addition, by connecting nanogenerators in series and parallel, the output voltage and current, respectively, can be increased as well[7].

Life-time testing of the fibre nanogenerator was performed at a motor speed of 80 r.p.m. for 1 min and 30 min of continuous operation. The ZnO nanowire array was robust and stood up well to the relative brushing between the two fibres, and no broken area was observed after 30 min of continuous sliding/brushing (Supplementary Fig. 8a, b). An SEM image recorded from the contact area shows that, just after 1 min operation, a small fraction of ZnO nanowires had been stripped off the fibres. These nanowires were either stuck in the gaps between gold-coated nanowires or laid on the surface (Supplementary Fig. 8c). However, after 30 min of continuous brushing, the fraction of stripped-off nanowires showed no significant increase (Supplementary Fig. 8d). This suggests that the nanowires that were stripped after the first 1 min (Supplementary Fig. 8c) were probably the loose ones in the arrays, which would be stripped off anyway at any stage of the experiment, but the remaining nanowires were bonded well to the substrate. This experiment further confirms that the TEOS infiltration had effectively improved the mechanical toughness and stability of the ZnO nanowires and the life-time of the fibre nanogenerator.

The textile-fibre-based nanogenerator has demonstrated the following innovative advances in comparison to the DC nanogenerators reported previously[6]. First, using ZnO nanowires grown on fibres, it is possible to fabricate flexible, foldable, wearable and robust power sources in any shape (such as a 'power shirt'). Second, the output electricity can be dramatically enhanced using a bundle of fibres as a yarn, which is the basic unit for fabrics. The optimum output power density from textile fabrics can be estimated on the basis of the data we have reported, and an output density of 20–80 mW per square metre of fabric is expected (see Supplementary Information). Third, the nanogenerator operates at low frequency, in the range of conventional mechanical vibration, footsteps and heartbeats, greatly expanding the application range of nanogenerators. Last, as the ZnO nanowire arrays were grown using chemical synthesis at 80 °C on a curved substrate, we believe that our method should be applicable to growth on any substrate, so the fields in which the nanogenerators can be applied and integrated may be greatly expanded. The near-term goal is to optimize the structure and design in order to improve the efficiency and total output power.

METHODS SUMMARY

The ZnO nanowires were grown radially around Kevlar 129 fibres using a hydrothermal approach. The as-grown ZnO nanowires were then chemically bonded to the fibre surface as well as to each other by coating with TEOS. The

double-fibre nanogenerator was assembled by entangling a fibre covered with as-grown nanowires around the other fibre covered with gold-coated nanowires. By fixing the two ends of one fibre, and sliding the other fibre back and forth, a relative brushing motion between the two fibres produces output current due to coupled piezoelectric–semiconducting properties. Short circuit current and open circuit voltage were recorded when the two fibres were slid with respect to each other.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions Z.L.W., X.W. and Y.Q. designed the experiments; Y.Q. and X.W. performed the experiments; and Z.L.W. and X.W. analysed the data and wrote the paper. All authors discussed the results and commented on the manuscript.

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LETTERS

Sound velocities of majorite garnet and the composition of the mantle transition region

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The composition of the mantle transition region, characterized by anomalous seismic-wave velocity and density changes at depths of ~400 to 700 km, has remained controversial. Some have proposed that the mantle transition region has an olivine-rich 'pyrolite' composition[1,2], whereas others have inferred that it is characterized by pyroxene- and garnet-rich compositions ('piclogite'), because the sound velocities in pyrolite estimated from laboratory data are substantially higher than those seismologically observed[3–5]. Although the velocities of the olivine polymorphs at these pressures (wadsleyite and ringwoodite) have been well documented, those of majorite (another significant high-pressure phase in the mantle transition region) with realistic mantle compositions have never been measured. Here we use combined *in situ* X-ray and ultrasonic measurements under the pressure and temperature conditions of the mantle transition region to show that majorite in a pyrolite composition has sound velocities substantially lower than those of earlier estimates, owing to strong nonlinear decreases at high temperature, particularly for shear-wave velocity. We found that pyrolite yields seismic velocities more consistent with typical seismological models than those of piclogite in the upper to middle parts of the region, except for the potentially larger velocity jumps in pyrolite relative to those observed at a depth of 410 km. In contrast, both of these compositions lead to significantly low shear-wave velocities in the lower part of the region, suggesting possible subadiabatic temperatures or the existence of a layer of harzburgite-rich material supplied by the subducted slabs stagnant at these depths.

It has been accepted that the upper mantle is composed of peridotite or pyrolite compositions with about 60% olivine and lesser amounts of pyroxenes (~30%) plus a small but significant (~10%) amount of Al-rich phases, such as spinel and garnet, depending on pressure[1]. Olivine transforms to wadsleyite at a pressure of ~13.5 GPa, and then to ringwoodite at ~18 GPa and decomposes to an assemblage of $MgSiO_3$-rich perovskite and (Mg,Fe)O rocksalt at ~24 GPa. In contrast, pyroxene progressively transforms to garnet structure with increasing pressure, forming an Al-deficient garnet (majorite) at pressures above ~16 GPa (ref. 6). Therefore, the mantle transition region (MTR) at depths between 410 km (~13.5 GPa) and 660 km (~24 GPa) is believed to be mainly composed of high-pressure forms of olivine and majorite, although small amounts of clinopyroxne and $CaSiO_3$-rich perovskite may be present at shallower and deeper regions of MTR, respectively[6–8].

Laboratory sound velocity measurements, however, have demonstrated that the velocities of wadsleyite (and ringwoodite) are substantially higher than those of olivine[5], yielding unacceptably large velocity contrasts across the 410-km seismic discontinuity and also velocities too high to match seismological models in pyrolite composition for the upper to middle parts of the MTR. In contrast, majorite has been shown to exhibit lower velocities relative to wadsleyite and ringwoodite[9–12], and provides better fits to seismological models if the proportion of olivine is reduced to 30–40%. So piclogite has been thought to yield velocities more consistent with those observed seismologically, although the uncertainty in laboratory measurements of the sound velocities has hindered firm conclusions[5,13–16] because none of these measurements has been made under MTR conditions. We recently expanded the pressure and temperature conditions for combined *in situ* X-ray and ultrasonic measurements towards those of the MTR[17]. Here we applied this method to majorite with a 'pyrolite minus olivine' composition[6] to address the chemical composition of MTR by combining the results on well-defined sound velocities of ringwoodite[17–20].

We conducted *in situ* X-ray and ultrasonic measurements under the pressure and temperature conditions of the MTR (Fig. 1a). Figure 1b shows a transmission electron microscope (TEM) image of the recovered sample after the run, showing that well-sintered and equilibrium textures formed, but no secondary phases, including no metallic iron. In contrast, we were unable to see any notable textural features on the polished sample using a scanning electron microscope (SEM), because the sample was fairly homogeneous and well-sintered with very small (<500 nm) grains. Powder X-ray diffraction measurement at the ambient condition for the sample demonstrated that all of the observed diffraction lines correspond to those of majorite (Supplementary Fig. 1), yielding lattice parameter $a = 11.580(1)$ Å, which is almost the same as the value before the ultrasonic run ($a = 11.582(1)$ Å). We note that a substantial decrease in lattice parameter is expected to occur if the majorite partially transforms to the low-pressure phase (clinopyroxene) or to the high-pressure phase ($CaSiO_3$-rich perovskite)[6].

Figure 1c and d and Supplementary Table 1 show the sound velocity changes in the present majorite with increasing pressure and temperature. Both the P- and S-wave velocities v_P and v_S increase with increasing pressure and decrease with increasing temperature, whereas the temperature effect on v_S is so large that at the highest pressure (~18 GPa) and temperature (1,673 K), v_S is substantially lower than at the ambient condition. Also, the rates of the velocity decreases in both v_P and v_S become more prominent at higher temperatures. A similar but less obvious nonlinear temperature dependence was also observed for $(Mg,Fe)_2SiO_4$ ringwoodite under corresponding pressure and temperature conditions[17].

Unit-cell volume changes of majorite with pressure and temperature are determined on the basis of *in situ* X-ray diffraction measurements. The X-ray density of majorite at each pressure and temperature can be determined by the volume data combined with the chemical composition of majorite (Fig. 1e and Supplementary Table 1), yielding a zero-pressure density of $\rho_0 = 3.605(1)\ g\,cm^{-3}$, which is in good agreement with the earlier result by quench method ($\rho_0 = 3.61\ g\,cm^{-3}$) (ref. 6). The isothermal bulk modulus derived from the compression data at room temperature

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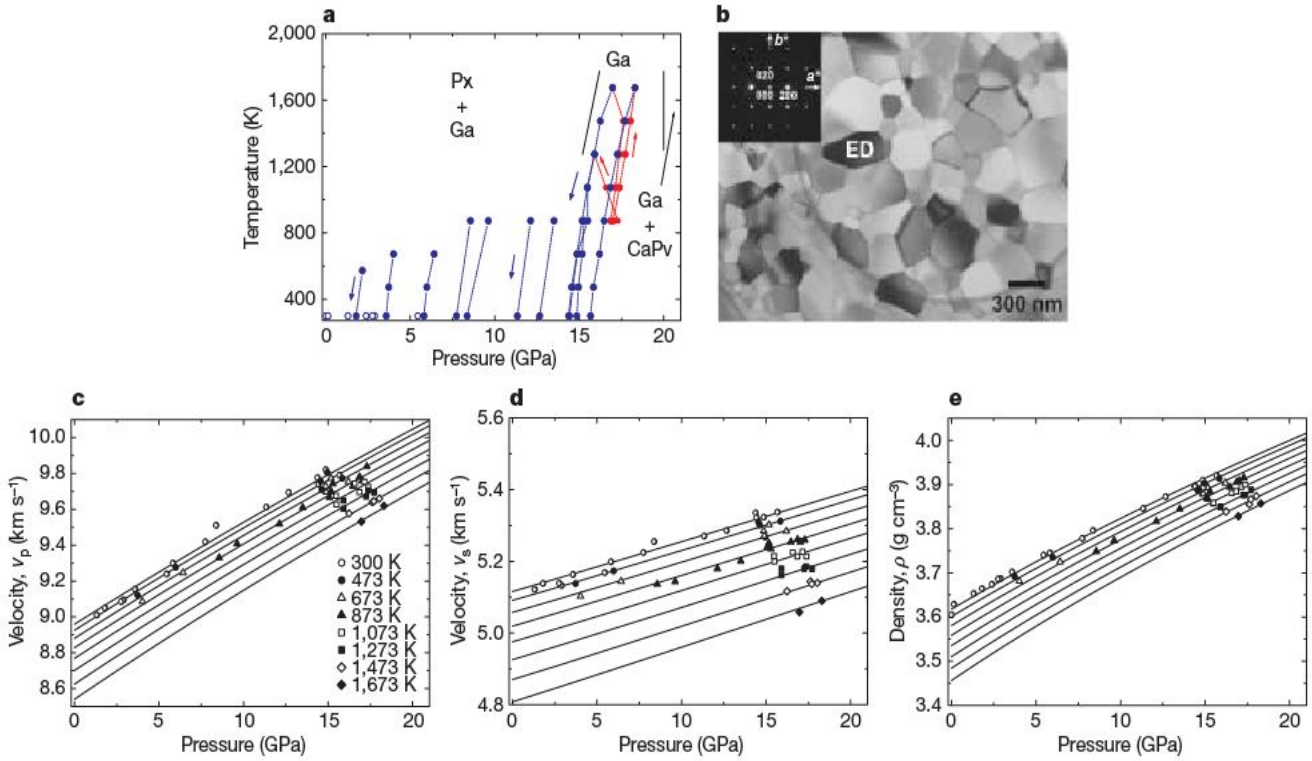

Figure 1 | Experimental conditions and results of the combined ultrasonic and *in situ* X-ray measurements on a polycrystalline majorite sample with pyrolite minus olivine composition. **a**, Pressure–temperature conditions of the combined *in situ* X-ray and ultrasonic measurements. The measurements were mostly made while decreasing temperature (blue), but some measurements in the stability field of majorite were made while increasing temperature (red). The open circles represent pressures where the measurements were made without heating the sample upon compression. Phase boundaries are based on a phase equilibrium study[6]. Px = clinopyroxene; Ga = majorite garnet; CaPv = $CaSiO_3$-rich perovskite. **b**, A TEM image of the sample recovered from the sound velocity measurements at high pressure and high temperature. The inset is a selected area electron diffraction pattern from the grain marked ED—a single crystal of majorite garnet. No evidence for the presence of other phases is obtained, and the crystals exhibit an equilibrium texture with grain sizes of typically 200–300 nm. **c**–**e**, P-wave velocity (**c**), S-wave velocity (**d**) and density (**e**) changes of majorite with pressure and temperature.

(K_{T0} = 162(1) GPa, assuming K_{T0}' = 4.2) is consistent with the bulk modulus K_{S0} = 164.4(5) GPa based on the ultrasonic measurements.

By a linear fitting of the longitudinal and shear moduli derived from the velocity data combined with the X-ray density data, we obtained the bulk and shear moduli K_0 and G_0 and their pressure and temperature derivatives (Table 1), which are in general comparable to those obtained for majorite with a composition of 50% $MgSiO_3$ and 50% $Mg_3Al_2Si_3O_{12}$ ($En_{50}Py_{50}$), using the Brillouin scattering method[11,12], although the dG/dP and dG/dT values we obtained are smaller. We also obtained the second temperature derivatives for our majorite by fitting a polynomial equation to the same data set, and calculated variations of the sound velocities with temperature at some pressures (16, 18 and 20 GPa) of the middle part of the MTR (Fig. 2). v_P and v_S and their temperature dependence in the present pyrolitic majorite are quite close to those of $En_{50}Py_{50}$ majorite at relatively low temperatures below ~1,000 K. However, these velocities, particularly v_S, based on the present data, exhibit a notable nonlinear decrease with temperature, as compared with those of $En_{50}Py_{50}$ majorite, which were extrapolated using the experimental data at relatively low (<1,073 K) temperatures[12].

Majorite garnet is suggested to have a temperature dependence of less than half ($dv_P/dT = -1.6 \times 10^{-4}$ km s^{-1} K^{-1} and $dv_S/dT = -1.1 \times 10^{-4}$ km s^{-1} K^{-1}) that of high-pressure forms of olivine ($dv_P/dT = -3.5 \times 10^{-4}$ km s^{-1} K^{-1}and $dv_S/dT = -3.8 \times 10^{-4}$ km s^{-1} K^{-1} for wadsleyite, $dv_S/dT = -3.0 \times 10^{-4}$ km s^{-1} K^{-1} and $dv_S/dT = -2.9 \times 10^{-4}$ km s^{-1} K^{-1} for ringwoodite) near the bottom of the MTR[12]. We obtained similarly small negative values ($dv_P/dT = -1.5 \times 10^{-4}$ km s^{-1} K^{-1} and $dv_S/dT = -1.2 \times 10^{-4}$ km s^{-1} K^{-1}) for our majorite from the low-temperature data at 18 GPa. However, because of the notable nonlinear nature of the temperature dependence, these temperature derivatives at high temperatures at this depth are calculated to be $dv_P/dT = -4.0 \times 10^{-4}$ km s^{-1} K^{-1} and $dv_S/dT = -3.1 \times 10^{-4}$ km s^{-1} K^{-1}, which are similar to those of the high-pressure forms of olivine. Therefore we suggest that the temperature variations in the MTR inferred from the lateral velocity heterogeneity combined with the elastic properties of only high-pressure forms of olivine would not be affected by the presence of majorite, in spite of the earlier study based on the results at lower temperatures[12]. The $R = d\ln v_S/d\ln v_P$ value[21] for our majorite is 1.5 for all the present temperatures at 18 GPa, which is higher than those of

Table 1 | Elastic parameters of majorite

Composition	K_0 (GPa)	G_0 (GPa)	dK/dP	dG/dP	dK/dT (GPa K^{-1})	dG/dT (GPa K^{-1})	d^2K/dT^2 (GPa K^{-2})	d^2G/dT^2 (GPa K^{-2})	Reference
Pyrolite minus olivine	164.4(5)	94.9(2)	4.24(6)	1.11(3)	−0.0129(8)	−0.0103(4)	NA	NA	This study*
Pyrolite minus olivine	164.2(5)	94.7(2)	4.22(5)	1.08(2)	−0.0091(16)	−0.0074(5)	$-6(3) \times 10^{-6}$	$-5(1) \times 10^{-6}$	This study**
En38Py62	171(5)	90(1)	6.2(5)	1.9(2)	NA	NA	NA	NA	Ref. 10
En50Py50	167(3)	90(2)	NA	NA	−0.0145(20)	−0.0082(10)	NA	NA	Ref. 12
En50Py50	167(3)	90(2)	4.2(3)	1.4(2)	NA	NA	NA	NA	Ref. 11
En80Py20	163(3)	88(2)	NA	NA	−0.0143(20)	−0.0083(10)	NA	NA	Ref. 12

NA, not available. *,**Linear and polynomial fits (including the second derivative of temperature $M = M_0 + dM/dP \times P + dM/dT \times (T - 300) + (1/2)d^2M/dT^2 \times (T - 300)^2$, where M represents the longitudinal and shear moduli, respectively).

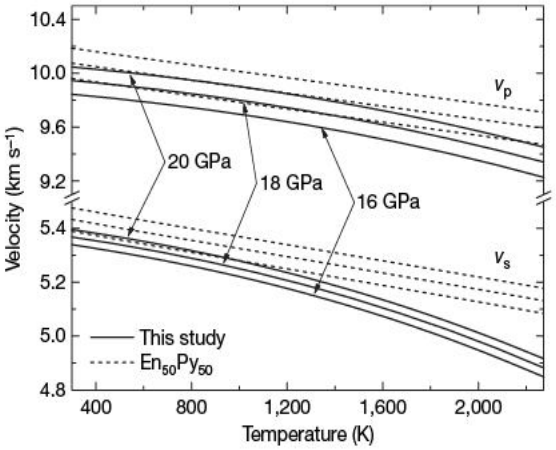

Figure 2 | Variations of sound velocities of majorite with temperature for the selected pressures in MTR. The solid lines show those derived from the present data, while the dotted lines are extrapolated using the elastic properties of majorite with a composition $En_{50}Py_{50}$, where En is $MgSiO_3$ and Py is $Mg_3Al_2Si_3O_{12}$ (ref. 12).

most high-pressure phases but is close to those seismologically constrained, whereas dv_P/dP and dv_S/dP are 0.049–0.052 and 0.014 km s^{-1} GPa^{-1} over the pressure range of the MTR.

We evaluated v_P and v_S for pyrolite and piclogite compositions, using elastic moduli and X-ray densities obtained for $(Mg_{0.91}Fe_{0.09})_2SiO_4$ ringwoodite[17] and those of our majorite along a geotherm[22] (Fig. 3). These velocities for the middle part of the MTR (~520–580 km), where only ringwoodite and majorite coexist in both of these mantle compositions[6,16], are tightly constrained within ~0.5%. We also estimated the velocity changes in pyrolite and piclogite in other regions of the MTR using our data and those of earlier studies on other high-pressure phases[15,17,23] in conjunction with the phase proportion data[6,7,16]. These calculations for the upper and lower parts of the MTR involve errors of ~2–3% owing to uncertainties in the mineral physics parameters.

For v_P, both pyrolite and piclogite compositions lead to the velocities consistent with those of the typical seismological models[24,25] throughout the MTR. v_S for pyrolite also agrees well with those of the seismological models in the upper half to middle parts of MTR, whereas piclogite yields substantially lower v_S in these regions. It should be noted that pyrolite yields larger velocity jumps at 410 km discontinuity than do the seismological models and piclogite. A corresponding sound velocity measurement on wadsleyite at the relevant pressure and temperature conditions is needed to address this issue, because the velocities of wadsleyite at temperatures of MTR may be lower than those estimated earlier, which yield smaller velocity jumps in pyrolite, consistent with the seismological models.

For the lower part of the MTR, both pyrolite and piclogite fail to explain the seismological v_S models, mainly because of the unexpectedly low v_S of majorite. The progressive formation of $CaSiO_3$-rich perovskite (which has high velocities) from the majorite phase may explain the discrepancy in these compositions, but direct measurement of this unquenchable phase under these pressure and temperature conditions is needed to confirm this.

There may be a low-temperature anomaly due to the presence of stagnant slabs near the 660 km depth[26], which should increase the average velocities in this region. The temperature dependence of the velocities for ringwoodite and majorite obtained in our study indicates that temperatures lower by about 400 K are required to match the seismological models. On the other hand, subducted slabs are generally assumed to be composed of a thin layer of oceanic crust underlain by a thicker residual harzburgite. This harzburgite should have olivine content and an Mg/(Mg+Fe) value substantially higher than those of the surrounding mantle, both of which increase the seismic velocities. Thus a combination of relatively low temperatures and the Mg- and olivine-rich nature of the stagnant slab may explain the apparent

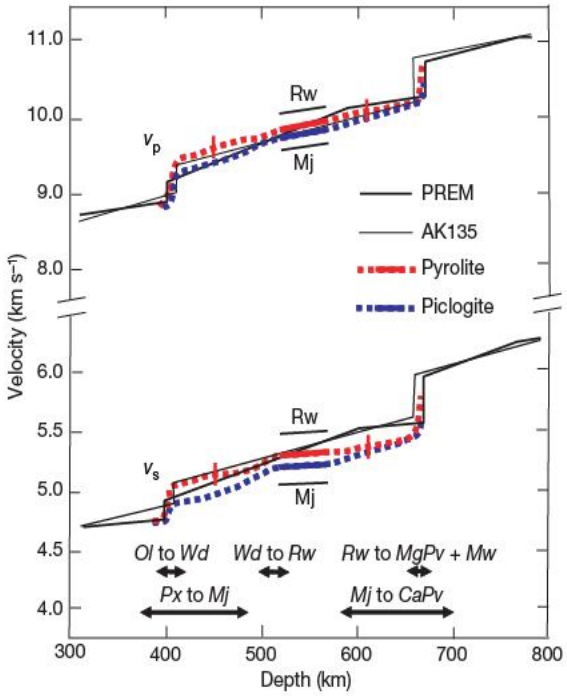

Figure 3 | A comparison of the sound velocities for pyrolite and piclogite compositions with representative seismological models in the MTR. Short black horizontal solid lines show the velocities of majorite (Mj, obtained in this study) and of ringwoodite (Rw, using the same method[17]). Thicker horizontal solid lines are for pyrolite (red) and piclogite (blue) calculated by combining these data and the mineral proportions defined by phase equilibrium studies[6,16]. The dotted red and blue lines are the estimated velocity changes using a combination of the phase equilibrium data[6–8,16] and the present and other elastic properties of relevant high-pressure phases[15,17,23]. Vertical red bars show typical errors in the velocity calculation for the upper and lower parts of the MTR (those based on the present study for the middle part are nearly equivalent to the thickness of the solid bars). Arrows indicate the depths at which phase transitions in major minerals take place in pyrolite[6]. Ol = olivine; Wd = wadsleyite; Py = pyroxene; MgPv = $MgSiO_3$ perovskite; Mw = magnesiowüstite. PREM and Ak135 are representative seismological models[24,25].

disagreement of the elastic velocities of pyrolite (and piclogite) and those of the seismological models in the lower part of the MTR.

METHODS

A polycrystalline sample of majorite with the 'pyrolite minus olivine' composition was synthesized using a multi-anvil apparatus at 18 GPa at 1,500 K under dry conditions. The hot-pressed rod sample was of pure majorite with grain sizes less than ~0.2 μm, and had a porosity of 0.5%. An electron microprobe analysis shows the sample to be homogeneous and to have composition SiO_2 = 50.60, TiO_2 = 0.60, Al_2O_3 = 11.14, Cr_2O_3 = 0.97, MgO = 22.32, CaO = 9.33, FeO = 3.02 and Na_2O = 0.75 (wt%), which is close to that reported in ref. 6.

Combined *in situ* X-ray and ultrasonic measurements were conducted using a 1,500-ton multianvil apparatus at SPring-8. The sample was surrounded by an NaCl sleeve and heated with a cylindrical platinum foil in a (Mg,Co)O pressure medium. The pressure was monitored by unit-cell volumes of NaCl and Au in a pellet of a mixture of NaCl+Au+BN, placed adjacent to the sample, using equations of state[27,28]. We used the NaCl scale as the primary pressure scale in the present analysis. The temperature was measured with a $W_{97}Re_3$–$W_{75}Re_{25}$ thermocouple, the hot junction of which was placed near the sample. The temperature uncertainty due to the gradient in the furnace and fluctuation of heating is within ±2% of the nominal value.

X-ray diffraction from the sample and the pressure markers was observed using an energy-dispersive system, and the sample length was measured with a high-resolution (1 pixel = ~2 μm) charge-coupled device (CCD) camera. Ultrasonic measurements were conducted while *in situ* X-ray observations were being made, and the travel times of both P- and S-waves were determined by a pulse–echo method using a transfer function technique[29]. The data acquisition time at each pressure and temperature point was typically 10 min. The overall uncertainties of the present velocity measurements are within ±0.5% in both v_P and v_S.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions T. Irifune directed the research project and wrote the manuscript. Y.H. did most of the experiments and analyses of the data with the help of T. Inoue, Y.K. and K.F. The TEM and XRD analyses of the recovered sample were conducted by H.O. and T. Inoue, respectively. All authors discussed the results and commented on the manuscript.

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LETTERS

Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation

Nancy B. Simmons[1], Kevin L. Seymour[2], Jörg Habersetzer[3] & Gregg F. Gunnell[4]

Bats (Chiroptera) represent one of the largest and most diverse radiations of mammals, accounting for one-fifth of extant species[1]. Although recent studies unambiguously support bat monophyly[2–4] and consensus is rapidly emerging about evolutionary relationships among extant lineages[5–8], the fossil record of bats extends over 50 million years, and early evolution of the group remains poorly understood[5,7–9]. Here we describe a new bat from the Early Eocene Green River Formation of Wyoming, USA, with features that are more primitive than seen in any previously known bat. The evolutionary pathways that led to flapping flight and echolocation in bats have been in dispute[7–18], and until now fossils have been of limited use in documenting transitions involved in this marked change in lifestyle. Phylogenetically informed comparisons of the new taxon with other bats and non-flying mammals reveal that critical morphological and functional changes evolved incrementally. Forelimb anatomy indicates that the new bat was capable of powered flight like other Eocene bats, but ear morphology suggests that it lacked their echolocation abilities, supporting a 'flight first' hypothesis for chiropteran evolution. The shape of the wings suggests that an undulating gliding–fluttering flight style may be primitive for bats, and the presence of a long calcar indicates that a broad tail membrane evolved early in Chiroptera, probably functioning as an additional airfoil rather than as a prey-capture device. Limb proportions and retention of claws on all digits indicate that the new bat may have been an agile climber that employed quadrupedal locomotion and under-branch hanging behaviour.

The Green River Formation of Wyoming has produced many spectacular fossils, including *Icaronycteris index*, widely regarded as the oldest and most primitive known bat[9]. *Icaronycteris* comes from the Fossil Butte Member of the Green River Formation, late early Eocene epoch, about 52.5 Myr ago[9,19,20]. Recently a new bat was discovered in the Fossil Butte Member that differs from *Icaronycteris* and other Eocene bats in being larger, having more primitive limb proportions and basicranium, and possessing well- developed claws on all wing digits. Because it cannot be assigned to any existing taxon, we here describe it as a new family, genus and species.

Order Chiroptera
Family Onychonycteridae fam. nov.
Onychonycteris finneyi gen. et sp. nov.

Etymology. *Onycho* (Greek): clawed; *nycteris* (Greek): bat. The genus name refers to the well-developed claws present on all hand digits. The specific epithet is given in honour of Bonnie Finney, who collected the holotype.

Holotype. ROM 55351A,B (Fig. 1 and Supplementary Figs 1–3), part and counterpart of a nearly complete, articulated skeleton including skull with lower jaws, found on 21 August 2003 at Finney Quarry, Lincoln County, Wyoming, USA.

Referred material. A specimen in a private collection also represents *O. finneyi* (Supplementary Fig. 4). First-generation casts of this specimen are housed in the American Museum of Natural History (AMNH 142467), in the University of Michigan, Museum of Paleontology (UM 12405), in the Royal Ontario Museum (ROM 55055) and at Fossil Butte National Monument (FOBU 9840).

Horizon and age. Both specimens are from the Sandwich Beds, Fossil Butte Member, Green River Formation, late early Eocene (Lostcabinian, Wasatchian Biochron Wa-7).

Diagnosis. Differs from all other known bats in having large claws on wing digits I, II and III, and small but distinct claws on digits IV and V. Cochlea and orbicular apophysis relatively small; stylohyal with small, rounded cranial tip; metacarpal formula (shortest to longest) I:II:III:IV:V; second phalanx longer than first phalanx in wing digits II–IV, first phalanx longer than the second in digits I and V; manubrium with keel-like ventral process; hind legs long and robust with complete fibula; feet with digit I shorter than digits II–V; calcar present; tail long.

Description and comparisons. *Onychonycteris finneyi* is a medium-sized bat that is substantially larger than most other known Eocene bats (Supplementary Table 1). The dentition is tribosphenic and resembles that of other Eocene chiropterans (Fig. 1b). The dental formula is I2/3, C1/1, P3/3, M3/3. In comparison with other bats including most Eocene taxa, *Onychonycteris* has a relatively small cochlea (Fig. 2). Unlike other Eocene bats[9], *Onychonycteris* has a malleus with blunt and rounded orbicular apophysis that is relatively small, and a stylohyal bone that lacks any paddle-like expansion of the cranial tip (Fig. 1b).

The axial skeleton of *Onychonycteris* is similar to *Icaronycteris* with 7 cervical, 12 thoracic, 7 lumbar and 12 or 13 caudal vertebrae. The ventral process of the manubrium forms a laterally compressed keel (Fig. 1c). Rib and vertebral fusions common in several bat lineages are absent in *Onychonycteris*. The scapula resembles that of *Icaronycteris*, although it is not possible to determine whether a dorsal articular facet is absent in *Onychonycteris* as in *Icaronycteris*.

The morphology of wing elements in *Onychonycteris* is similar to that in other bats. As in *Icaronycteris* and *Archaeonycteris*, the trochiter (= greater tuberosity) of the humerus extends to the level of the proximal edge of the head, which is round in medial view. Each wing digit has three ossified phalanges. Digits I–III terminate in large claws, with smaller claws on digits IV–V. This pattern is unique among bats. With the exception of some pteropodids that retain a claw on digit II, all extant bats lack claws on digits II–V. *Icaronycteris* has a well-developed claw on digit II, but digits III–IV terminate in tiny ossifications that are not claw shaped. *Archaeonycteris* has a claw on digit II but lacks ossified third phalanges on the remaining digits.

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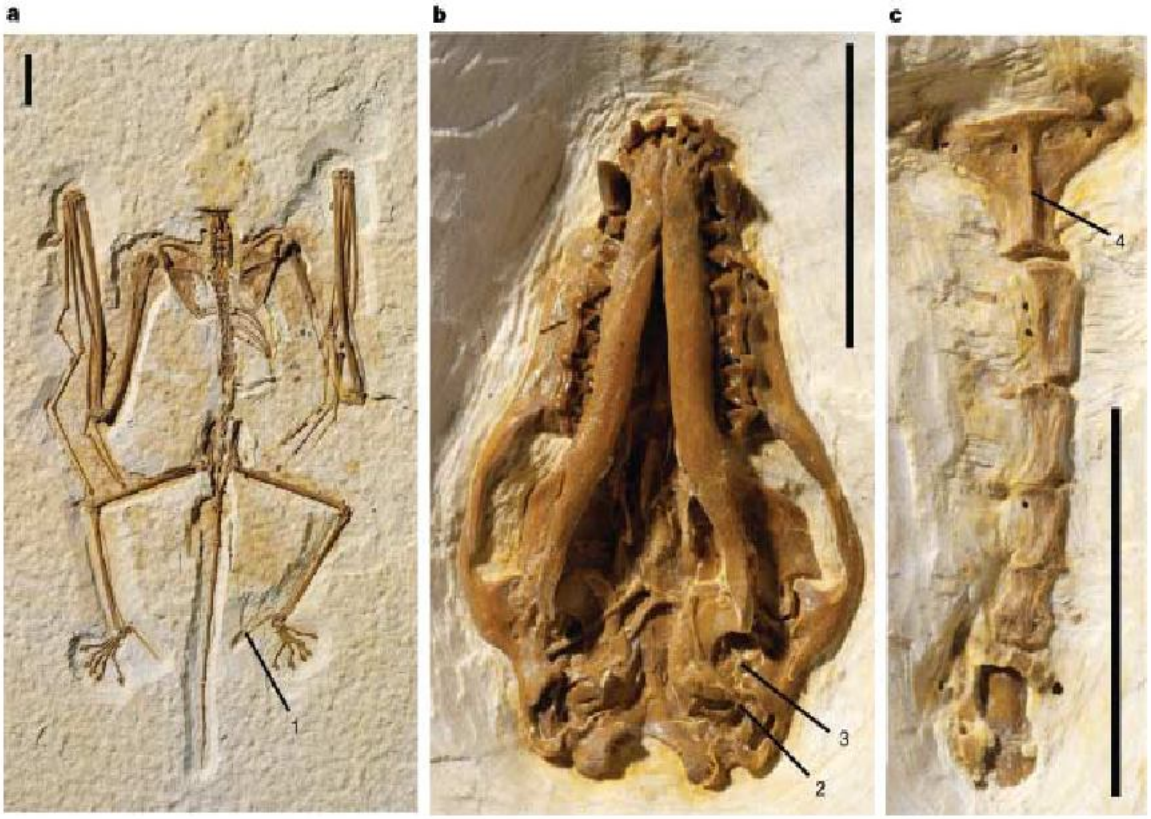

Figure 1 | Holotype of *Onychonycteris finneyi* (ROM 55351A). **a**, Skeleton in dorsal view. **b**, Skull in ventral view. **c**, Sternum in ventral view. Scale bars, 1 cm. All elements are preserved on a single slab with the skeleton exposed on one side, and the skull and sternum on the reverse. The counter-part slab (ROM 55351B, not shown) preserves impressions of parts of the dorsal aspect of the skeleton. Features labelled: 1, calcar; 2, cranial tip of stylohyal; 3, orbicular apophysis of malleus; 4, keel on manubrium of sternum.

Palaeochiropteryx and *Hassianycteris* lack ossified third phalanges on digits II–IV. The metacarpal formula (I:II:III:IV:V) in *Onychonycteris* is unique among Eocene bats. The second phalanx is longer than the first phalanx in digits II–IV, whereas the first phalanx is longer than the second in digits I and V. In this respect *Onychonycteris* resembles *Tachypteron*[21]. The wings of *Onychonycteris* were relatively short and broad with a very low aspect ratio index of 1.74, a low wingtip area ratio of 0.51–0.53, and a low wingtip length ratio of 0.77 (Supplementary Table 2).

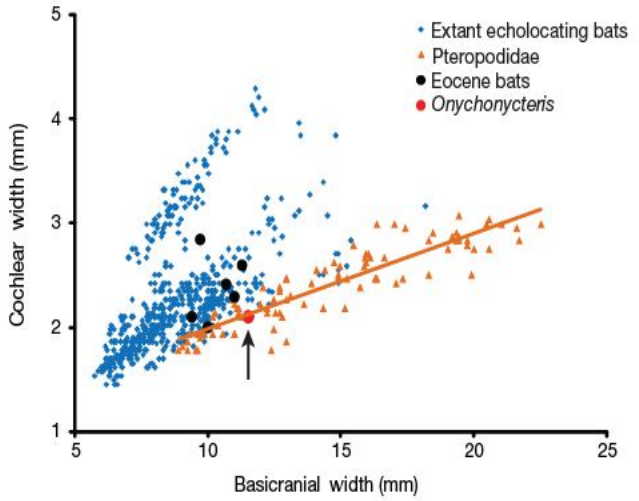

Figure 2 | Relative sizes of cochlea in extant and fossil bats. Cochlear size is known to be correlated with echolocation behaviour; non-echolocating bats (Pteropodidae) tend to have smaller cochleae than bats that use laryngeal echolocation (Rhinolophoidea, Emballonuroidea, Noctilionoidea and Vespertilionoidea)[9,22–24]. *Onychonycteris* (red circle with arrow) has a cochlea that is similar in size to those seen in Pteropodidae and smaller than observed in most echolocating bats. *Icaronycteris* (black circle on Pteropodidae regression line) has a similarly small cochlea. In contrast, other Eocene bats (*Archaeonycteris*, *Palaeochiropteryx*, *Hassianycteris*, *Tachypteron* and *Tanzanycteris*; black circles) have proportionately larger cochlea. See Supplementary Figs 7–9 for additional information.

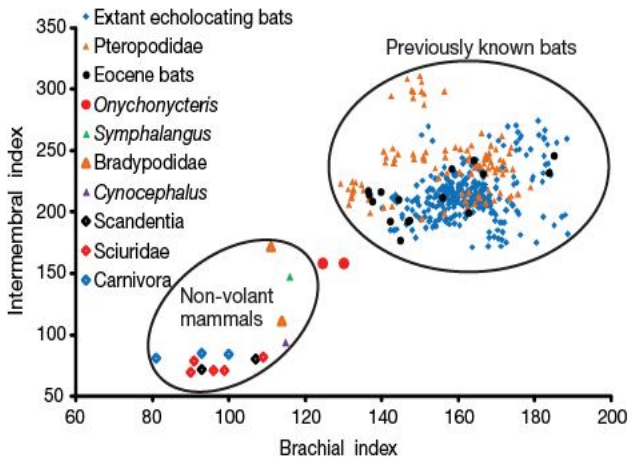

Figure 3 | Limb proportions in *Onychonycteris* compared with those of other bats and selected non-volant mammals. Intermembral index, defined as 100 × (radius length + humerus length)/(femur length + tibia length), describes the relative proportions of the forelimb and hindlimb; brachial index, defined as 100 × radius length/humerus length, describes the relative length of the forearm with respect to the upper arm. In overall limb proportions, *Onychonycteris* (red circles) falls midway between previously known bats and forelimb-dominated mammals that climb but do not fly (for example, Bradypodidae, *Cynocephalus* and *Symphalangus*). Typical scansorial mammals (for example, Scandentia, Sciuridae and Carnivora) have lower indices. Eocene bats indicated by black circles include *Icaronycteris*, *Archaeonycteris*, *Palaeochiropteryx* and *Hassianycteris*.

The limb proportions of *Onychonycteris* are unique among bats, being intermediate between all other known bats and forelimb-dominated non-volant mammals (Fig. 3). Elongation of the radius is less pronounced than in other bats, and the hindlimb is proportionally longer. The pelvis of *Onychonycteris* resembles that of other bats and the hindlimbs are rotated outwards as in all known chiropterans[2].

The feet of *Onychonycteris* are similar to those of other bats except that the overall length of the first digit is shorter than the other digits. In this respect *Onychonycteris* resembles *Icaronycteris* rather than extant bats, in which all five toes are subequal in length. The holotype of *Onychonycteris* has a relatively elongate calcar, a pattern common to many bats including *Palaeochiropteryx* and *Hassianycteris*. A calcar has never been observed in *Icaronycteris* or *Archaeonycteris*, but this may be an artefact of preservation.

Phylogenetic analyses based on morphological data indicate that *Onychonycteris* is the most basal bat presently known and is the sister group of the clade including *Icaronycteris* and all other extant and extinct bat lineages (Supplementary Fig. 5). Because morphology-based phylogenies of extant bats conflict with those based on gene sequences[4–9], we also conducted a phylogenetic analysis with the use of a molecular scaffold. The topology of the resulting tree (Fig. 4 and Supplementary Fig. 6) confirms the basal position of *Onychonycteris* within Chiroptera.

The evolution of flapping flight and laryngeal echolocation in bats has been the subject of debate. Three competing hypotheses have been proposed to explain the sequence of steps that lead to these key adaptations: 'flight-first', 'echolocation-first' and the 'tandem-development' hypothesis[4,5,7–9,12–17]. Basicranial morphology and stomach contents suggest that at least six Eocene bat taxa (*Icaronycteris*, *Archaeonycteris*, *Palaeochiropteryx*, *Hassianycteris*, *Tachypteron* and *Tanzanycteris*) were echolocating bats[9,21–25]. Recent phylogenetic analyses including Eocene bats have either concluded that laryngeal echolocation is primitive for Chiroptera[4,5,8] or could not rule out that possibility[7]. The basicranial region of *Onychonycteris* indicates that it has a relatively small cochlea similar to that seen in non-echolocating bats (Pteropodidae) but smaller than those found in most echolocating taxa (Fig. 2). *Icaronycteris* and *Archaeonycteris* have cochleae that also fall into the zone of overlap between echolocating and non-echolocating bats, but these taxa share two additional derived features linked with echolocation[9,23]: an enlarged orbicular apophysis on the malleus, and a stylohyal element with an expanded, paddle-like cranial tip, both of which are absent in *Onychonycteris* (Fig. 1b). In the absence of these traits, there is no unambiguous evidence that *Onychonycteris* was capable of laryngeal echolocation.

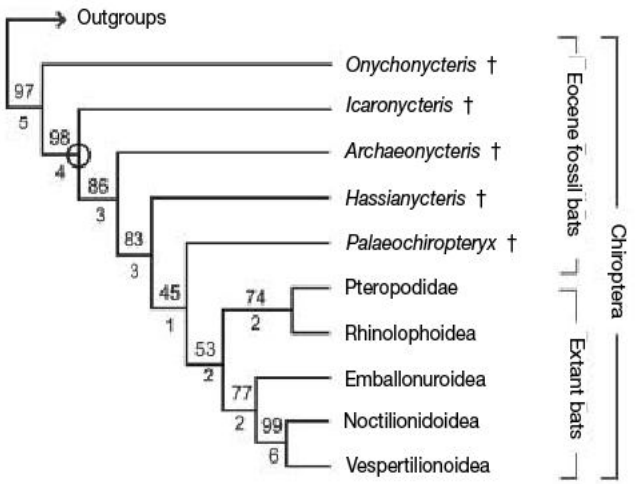

Figure 4 | Phylogenetic position of *Onychonycteris* and other Eocene fossils with respect to extant bat lineages. Relationships of fossil taxa to extant families (here grouped into superfamilies) were constrained by using a backbone scaffold tree derived from molecular studies. Numbers above branches are bootstrap values; numbers below branches are Bremer values. Derived features present in *Icaronycteris*, *Archaeonycteris*, and other extinct and extant bats (clade defined by node circled on tree) but absent in *Onychonycteris* include the following: enlarged orbicular apophysis on the malleus; stylohyal element with an expanded, paddle-like cranial tip; enlarged cochlea; ribs with posterior laminae; claws absent on forelimb digits III–V.

Onychonycteris was clearly capable of powered flight, on the basis of the morphology of the manus, the shape of the rib cage, the faceted infraspinous fossa of the scapula, the robust clavicle and the keeled sternum, all of which are derived features intimately associated with the chiropteran flight apparatus[9,15,26]. This suggests that powered flight evolved early in the bat lineage, with echolocation evolving later (after the divergence of *Onychonycteris*), thus supporting the 'flight-first' hypothesis for chiropteran evolution. The conclusion that flight evolved before echolocation in the chiropteran lineage is consistent with studies of flight, ventilation and echolocation in living bats, which have suggested that echolocation may be too energetically costly to be sustained in the absence of flight[13,14].

The low-aspect-ratio wings and relatively short and small wingtips of *Onychonycteris* are similar to those of extant mouse-tailed bats (Rhinopomatidae; Supplementary Fig. 10), which have an unusual undulating flight style in which fluttering is alternated with gliding[10,12,27,28]. In contrast, most extant microbats do not commonly glide[10]. On anatomical and aerodynamic grounds, gliding has been proposed as a probable intermediate step in the development of powered flapping flight in bats from a non-flying ancestry[9,12,15,27,28]. An undulating gliding–fluttering flight style reduces energy costs at most flight speeds and permits an animal to fly slowly most economically[29]. This flight style may represent both a functional and an evolutionary intermediate between gliding and continuous flapping flight in the chiropteran lineage.

The discovery of *Onychonycteris* indicates that claws on digits III–V were lost after powered flight evolved. The limb proportions indicate that elongation of the forearm (radius + ulna) and hand bones (metacarpals + phalanges) continued after powered flight evolved, and the very-low-aspect-ratio wings of *Onychonycteris* suggest that higher-aspect-ratio wings (including the long, narrow wings seen in Eocene forms such as *Hassianycteris*[11,12] and *Tachypteron*[21]) were derived later in chiropteran evolution.

Reduction in the relative size of the hind limbs also continued after the evolution of powered flight. The limb proportions, the robust fibula and the retention of wing claws seen in *Onychonycteris* suggest that it was probably capable of more agile non-volant locomotion (climbing either along tree branches or under them) than most other bats, including other Eocene taxa, and may have incorporated quadrupedal locomotion[30] and under-branch hanging[15] into its locomotory and roosting behaviour.

The calcar is a neomorphic element that supports the uropatagium (tail membrane) in most extant bats[18,26]. The presence of a long calcar in *Onychonycteris* indicates that the uropatagium, used by many bats to capture prey in flight[9,26], was present in the earliest bats, *contra* previous reconstructions[9,11,12,18]. Because aerial hawking for insects requires sophisticated echolocation capabilities[9,14,16,17,24] that were seemingly beyond the abilities of *Onychonycteris*, the uropatagium may instead have evolved as an additional airfoil to increase lift and reduce wing loading during powered flight[10,26] or to help with turning and braking[26], and was only later incorporated into the aerial insect capture apparatus by echolocating bats.

The morphology of the dentition suggests that *Onychonycteris* was insectivorous, like other Eocene bats. Lacking laryngeal echolocation, *Onychonycteris* probably detected its prey by vision, olfaction or passive auditory cues. It is not known whether the ancestors of bats were nocturnal, diurnal or crepuscular[14]. Living pteropodids, which forage and navigate while flying at night by using vision rather than echolocation, are characterized by enlarged eye orbits, and a diurnal

bat ancestor might be expected to have orbits intermediate in size between those of echolocating bats and pteropodids[14]. Unfortunately, orbital size in *Onychonycteris* cannot be estimated because of post-mortem crushing of both known specimens.

METHODS SUMMARY

Skeletal measurements were made with standard methods. Cochlear and basicranial widths were determined from X-ray microradiographs and, for *Onychonycteris*, measurements of external features of the basicranium. Phylogenetic analyses were based on parsimony analyses of a morphological data set of 207 characters scored in all extant bat families and four Eocene fossil genera. Five outgroups (*Tupaia*, *Cynocephalus*, *Erinaceus*, *Sus* and *Felis*) were included to root trees. The data were analysed with heuristic parsimony algorithms, and clade support was assessed with bootstrap and decay methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Information Morphological data used in phylogenetic analyses are deposited in MorphoBank and can be obtained at http://morphobank.geongrid.org/permalink/?P104. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to N.S. (simmons@amnh.org).

LETTERS

Chaos in a long-term experiment with a plankton community

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Mathematical models predict that species interactions such as competition and predation can generate chaos[1–8]. However, experimental demonstrations of chaos in ecology are scarce, and have been limited to simple laboratory systems with a short duration and artificial species combinations[9–12]. Here, we present the first experimental demonstration of chaos in a long-term experiment with a complex food web. Our food web was isolated from the Baltic Sea, and consisted of bacteria, several phytoplankton species, herbivorous and predatory zooplankton species, and detritivores. The food web was cultured in a laboratory mesocosm, and sampled twice a week for more than 2,300 days. Despite constant external conditions, the species abundances showed striking fluctuations over several orders of magnitude. These fluctuations displayed a variety of different periodicities, which could be attributed to different species interactions in the food web. The population dynamics were characterized by positive Lyapunov exponents of similar magnitude for each species. Predictability was limited to a time horizon of 15–30 days, only slightly longer than the local weather forecast. Hence, our results demonstrate that species interactions in food webs can generate chaos. This implies that stability is not required for the persistence of complex food webs, and that the long-term prediction of species abundances can be fundamentally impossible.

The discovery by May in the 1970s that simple population models may generate complex chaotic dynamics[1,2] triggered heated debate and caused a paradigm shift in ecology. Since May's findings, mathematical models have shown that chaos can be generated by a plethora of ecological mechanisms, including competition for limiting resources[6,8], predator–prey interactions[3,5] and food-chain dynamics[4,7]. In contrast to the overwhelming theoretical attention, convincing empirical evidence of chaos in real ecosystems is rare[13]. What could explain the paucity of empirical support? It might be that chaos is a rare phenomenon in natural ecosystems, for instance because food webs contain many weak links between species, which may stabilize food-web dynamics[14,15]. Alternatively, one might argue that there is a lack of suitable data to test for chaos in food webs. For instance, external variability (for example, weather fluctuations) may obscure the role of intrinsic species interactions. In principle, laboratory experiments provide ideal conditions to obtain high-resolution data in a constant environment. Chaos has so far been demonstrated experimentally for a few single species[9,10], a three-species food web[11] and nitrifying bacteria in a wastewater bioreactor[12]. Thus far, however, laboratory studies have not considered the natural complexity of real food webs, and the time span of experiments has often been too short to detect chaos in a rigorous manner.

Here, we analyse a time series of a plankton community isolated from the Baltic Sea. The plankton community was cultured in a laboratory mesocosm under constant external conditions for more than eight years[16]. In total, two nutrients (nitrogen and phosphorus), one detritus pool and ten different functional groups were distinguished (Fig. 1a). The phytoplankton was divided into picophytoplankton, nanophytoplankton and filamentous diatoms. The herbivorous zooplankton was classified into protozoa, rotifers and calanoid copepods. The rotifers and protozoa were grazed by cyclopoid copepods. The microbial loop was represented by heterotrophic bacteria and two groups of detritivores: ostracods and harpacticoid copepods. The abundances of these functional groups were counted twice a week. Our analysis covers a period of 2,319 days, which yielded 690 data points per functional group. Because most species in this food web have generation times of only a few days, the time series spanned hundreds to thousands of generations per species. We performed several analyses to investigate the dynamics of this food web.

First, the time series showed fluctuations in species abundances over several orders of magnitude, despite constant external conditions (Fig. 1). Spectral analysis revealed that the fluctuations covered a range of different periodicities (see Supplementary Information). In particular, picophytoplankton, rotifers and calanoid copepods seemed to fluctuate predominantly with a periodicity of about 30 days, suggestive of coupled phytoplankton–zooplankton oscillations. Periodicities of about 30 days are consistent with model predictions of phytoplankton–zooplankton oscillations[17], and have been observed in earlier laboratory experiments with phytoplankton and zooplankton species[18].

Second, a closer look at the species fluctuations revealed several striking patterns (Table 1). Peaks of picophytoplankton, nanophytoplankton and filamentous diatoms alternated with little or no overlap (Fig. 1d), and picophytoplankton and nanophytoplankton concentrations were negatively correlated (Table 1), indicative of competition between the phytoplankton groups. Predator–prey interactions could also be discerned. We found negative correlations of picophytoplankton with protozoa, and of nanophytoplankton both with rotifers and calanoid copepods (Table 1). This indicates that protozoa fed mainly on picophytoplankton, whereas rotifers and calanoid copepods fed mainly on larger nanophytoplankton, consistent with the structure of the food web (Fig. 1a). Conversely, the positive correlation of picophytoplankton with calanoid copepods may point at indirect mutualism between prey species and the predators of their competitors (that is, 'the enemy of my enemy is my friend'). Other striking patterns included the negative correlation between bacteria

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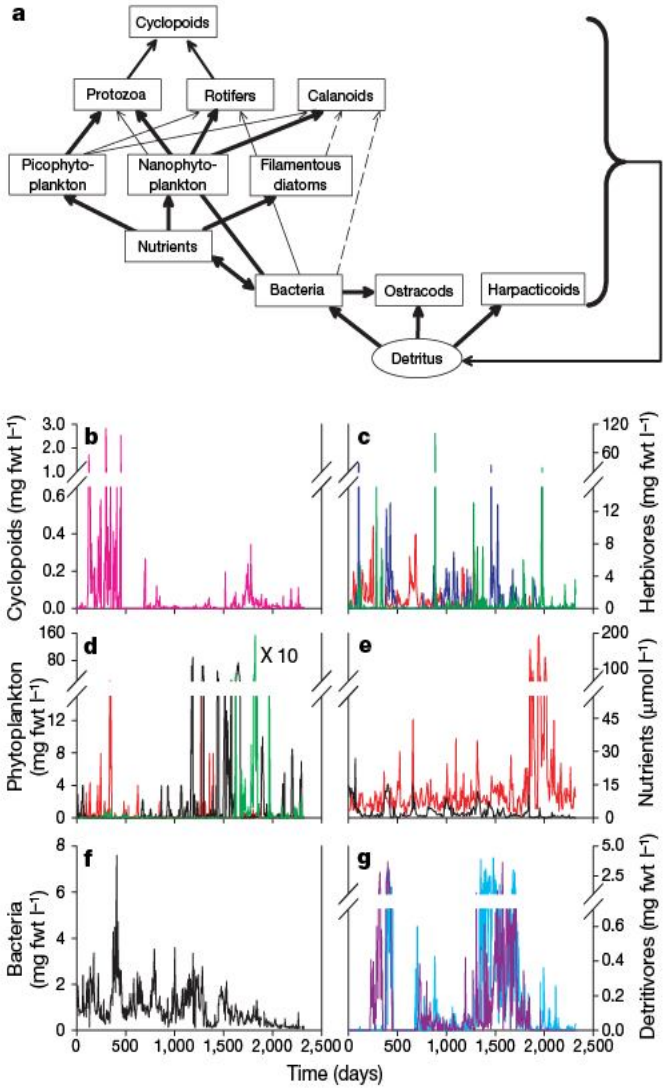

Figure 1 | Description of the plankton community in the mesocosm experiment. **a**, Food-web structure of the mesocosm experiment. The thickness of the arrows gives a first indication of the food preferences of the species, as derived from general knowledge of their biology. **b–g**, Time series of the functional groups in the food web (measured as freshweight biomass). **b**, Cyclopoid copepods; **c**, calanoid copepods (red), rotifers (blue) and protozoa (dark green); **d**, picophytoplankton (black), nanophytoplankton (red) and filamentous diatoms (green); note that the diatom biomass should be magnified by 10; **e**, dissolved inorganic nitrogen (red) and soluble reactive phosphorus (black); **f**, heterotrophic bacteria; **g**, harpacticoid copepods (violet) and ostracods (light blue).

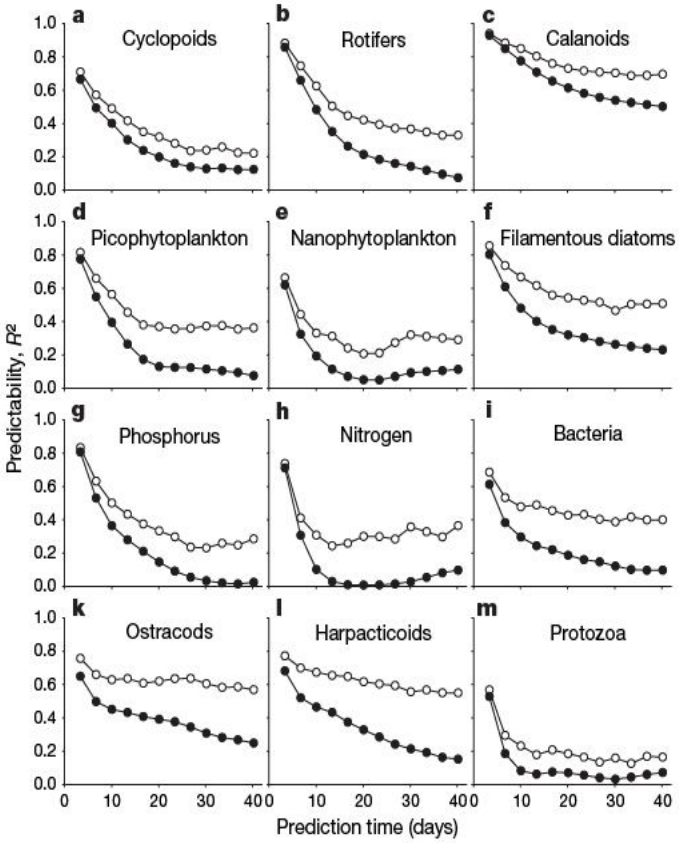

Figure 2 | Predictability of the species decreases with increasing prediction time. The predictability is quantified as the coefficient of determination R^2 between predicted and observed data. Already after a few time steps, predictions by the nonlinear neural network model (open circles) were significantly better than predictions by the best-fitting linear model (filled circles) (see Supplementary Information for further details). **a**, Cyclopoid copepods; **b**, rotifers; **c**, calanoid copepods; **d**, picophytoplankton; **e**, nanophytoplankton; **f**, filamentous diatoms; **g**, soluble reactive phosphorus; **h**, dissolved inorganic nitrogen; **i**, bacteria; **k**, ostracods; **l**, harpacticoid copepods; **m**, protozoa.

and ostracods, and the positive correlation between bacteria and phosphorus. Although our interpretation of these correlation patterns is somewhat speculative, they correspond with the trophic links in the food web. This shows that the observed fluctuations in species abundances were largely driven by species interactions in the food web, not by external forcing.

Third, we investigated the long-term predictability of the food-web dynamics. The predictability of a deterministic non-chaotic system with uncorrelated noise (for example, a limit cycle with sampling error) remains constant in time, whereas the predictability of chaotic systems decreases in time[19]. We fitted the time series to a neural network model[20] to generate predictions at different time intervals. For short-term forecasts of only a few days, most species had a high predictability of $R^2 = 0.70 - 0.90$ (Fig. 2). However, the predictability of the species was much reduced when prediction times were

Table 1 | Correlations between the species in the food web

	Bacteria	Harpacticoid copepods	Ostracods	Nitrogen	Phosphorus	Picophytoplankton	Nanophytoplankton	Rotifers	Protozoa	Calanoid copepods
Bacteria	1	0.03	**−0.24*****	0.05	**0.19*****	0.03	**−0.17*****	**0.30*****	−0.17	**0.22*****
Harpacticoid copepods		1	**0.17****	**−0.12***	0.09	−0.04	−0.03	0.02	0.14	−0.04
Ostracods			1	**−0.16*****	−0.06	−0.04	0.01	−0.03	**0.19***	−0.04
Nitrogen				1	0.08	−0.00	−0.02	−0.04	0.02	0.04
Phosphorus					1	−0.04	0.03	**0.10***	−0.11	−0.09
Picophytoplankton						1	**−0.17*****	−0.03	**−0.22****	**0.26*****
Nanophytoplankton							1	**−0.19*****	0.15	**−0.14***
Rotifers								1	−0.02	−0.10
Protozoa									1	n.a.
Calanoid copepods										1

Table entries show the product–moment correlation coefficients, after transformation of the data to stationary time series (see Methods). Significance tests were corrected for multiple hypothesis testing by calculation of adjusted P values using the false discovery rate[27]. Significant correlations are indicated in bold: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.a., not applicable. The correlation between calanoid copepods and protozoa could not be calculated, because their time series did not overlap. Filamentous diatoms and cyclopoid copepods were not included in the correlation analysis, because their time series contained too many zeros.

extended to 15–30 days. This is a characteristic feature of chaos, where short-term predictability is high, whereas the predictability decreases when making forecasts further into the future. However, decreasing predictability can also occur in linear (and therefore non-chaotic) systems exposed to stochastic perturbations. We therefore tested[19] whether the predictions of the nonlinear neural network model were significantly better than the predictions generated by the best-fitting linear model. Already after a few time steps, the nonlinear model yielded significantly higher predictabilities than the corresponding linear model for all species in the food web (Fig. 2; see Supplementary Information for the statistics). These findings demonstrate that (1) the predictability of the species abundances in the food web decreased in time, and (2) there was a strong nonlinear deterministic component in the food-web dynamics.

Fourth, we calculated the Lyapunov exponent, the hallmark of chaos in nonlinear systems. The dominant Lyapunov exponent λ is a measure of the rate of convergence or divergence of nearby trajectories[21]. Negative Lyapunov exponents indicate that nearby trajectories converge, which is representative of stable equilibria and periodic cycles. Conversely, positive Lyapunov exponents indicate divergence of nearby trajectories, which is representative of chaos. We used two different methods to calculate the Lyapunov exponent: a direct method and an indirect method.

The direct method started with a reconstruction of the attractor by time-delay embedding of each time series[21–23]. Exponential divergence (or convergence) of trajectories was calculated from nearby state vectors in the reconstructed state space[24]. The results show that the distance between initially nearby trajectories increased over time, and reached a plateau after about 20–30 days (Fig. 3). This matches the time horizon of 15–30 days obtained from the predictability estimates (Fig. 2). Lyapunov exponents were calculated from the initial slope of the exponential divergence, by using linear regression. This yielded significantly positive Lyapunov exponents of strikingly similar value for all species (Fig. 3; mean $\lambda \approx 0.057$ per day, s.d. = 0.005 per day, $n = 9$). This gives much confidence that the species in the food web were all fully connected, and that their population dynamics were governed by the same chaotic attractor.

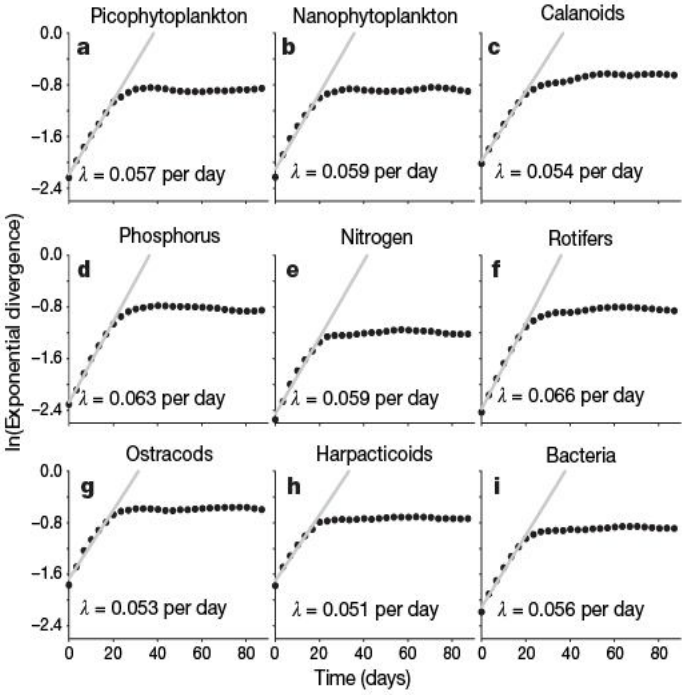

Figure 3 | Exponential divergence of the trajectories. The Lyapunov exponent (λ) is calculated as the initial slope of the ln-transformed exponential divergence versus time, as estimated by linear regression (grey line). All Lyapunov exponents were significantly different from zero (linear regression: $P < 0.001$, $n = 6$ or 7 depending on the species).
a, Picophytoplankton; **b**, nanophytoplankton; **c**, calanoid copepods; **d**, soluble reactive phosphorus; **e**, dissolved inorganic nitrogen; **f**, rotifers; **g**, ostracods; **h**, harpacticoid copepods; **i**, bacteria. Exponential divergence could not be calculated for filamentous diatoms, protozoa, and cyclopoid copepods, because their time series contained too many zeros.

Direct methods cannot distinguish trajectory divergence caused by chaos from trajectory divergence due to noise[9,20]. Therefore, we also applied an indirect method, which calculates the Lyapunov exponent from a deterministic model. While indirect methods are not affected by noise, they rely on the assumption that the deterministic model provides an adequate representation of the system's deterministic skeleton. In our case, the model structure again followed the trophic structure of the food web (Fig. 1a). The model was used to calculate trajectory divergence at each time step by evaluation of the jacobian matrix (see Supplementary Information for details). This indirect method yielded a global Lyapunov exponent of $\lambda = 0.04$ per day, characterizing the divergence of trajectories across the entire food web. We ran a bootstrap procedure based on 1,000 replicates to estimate the uncertainty of this value (see Supplementary Information). A one-sided confidence interval at the 95% confidence level yielded a lower bound of $\lambda = 0.03$ per day. This confirmed that the Lyapunov exponent was significantly positive, and that this positive value was not due to noise.

In total, our analysis revealed several signatures of chaos. Despite constant external conditions, the food web showed strong fluctuations in species abundances that could be attributed to different species interactions. We found high predictability in the short term, reduced predictability in the long term, and significantly positive Lyapunov exponents. This shows that the population dynamics in the food web were characterized by exponential divergence of nearby trajectories, which provides the first experimental demonstration of chaos in a complex food web.

Compared with other systems, the time horizon for the predictability of our plankton community (15–30 days) is only slightly longer than the time horizon for the local weather forecast (about two weeks[25]). Lyapunov exponents were smaller in our plankton community ($\lambda = 0.03$–0.07 per day) than in recent experiments with microbial food webs[11,12] ($\lambda = 0.08$–0.20 per day). This might indicate that our plankton was 'less chaotic'. Alternatively, these differences in Lyapunov exponents might be attributed to differences in generation times, because most phytoplankton and zooplankton species in our experiment have longer generation times than the bacteria and ciliates used in these microbial food webs. Because the time horizon is inversely proportional to the Lyapunov exponent[21], this suggests that the time horizon for the predictability of chaotic food webs scales with the generation times of the organisms involved.

Our findings have important implications for ecology and ecosystem management. First, our data illustrate that food webs can sustain strong fluctuations in species abundances for hundreds of generations. Apparently, stability is not required for the persistence of complex food webs. Second, non-equilibrium dynamics in food webs affect biodiversity and ecosystem functioning. For instance, fluctuations on timescales of 15–30 days, as observed in our experiment, offer a suitable range of temporal variability to promote species coexistence in plankton communities[26]. Hence, our results support the theoretical prediction that chaotic fluctuations generated by species interactions may contribute to the unexpected biodiversity of the plankton[6], which provides a solution for one of the classic paradoxes in ecology known as the paradox of the plankton. Third, chaos limits the predictability of species abundances. In our experimental food web, predictability was lost on a timescale of 15–30 days, which corresponds to 5 to 15 plankton generations depending on the species. Because many other food webs have a similar structure of plants, herbivores, carnivores and a microbial loop, it is tempting to suggest that the observed loss of predictability in 5 to 15 generations is likely to apply to many other food webs as well.

METHODS SUMMARY

The mesocosm consisted of a cylindrical plastic container (74 cm high, 45 cm diameter), which was filled with a 10 cm sediment layer and 90 l of water from the Baltic Sea. This inoculum provided all species in the food web. The mesocosm was maintained in the laboratory at a temperature of about 20 °C, a salinity of about 9‰, incident irradiation of 50 μmol photons $m^{-2} s^{-1}$ (16:8 h light:dark cycle) and constant aeration. Species abundances were measured twice a week, whereas nutrients were measured weekly.

We interpolated each time series to obtain data with equidistant time intervals of 3.35 days. The interpolated time series were subsequently transformed to stationary time series with mean zero and standard deviation of 1. Long sequences of zero values were removed from the analysis.

We calculated the predictabilities of the species by fitting a neural network model to the time series, following ref. 20. For each species, the neural network predictions were based on the observed population abundances of the species itself and of those species with which it had a direct link in the food web (Fig. 1a).

We used two different methods to calculate the Lyapunov exponent. The direct method was based on attractor reconstruction by time-delay embedding of each time series[23,24]. We chose an embedding dimension of six and a time delay of one time step (see Supplementary Information). This direct method yielded Lyapunov exponents for each species separately. The consistency of these Lyapunov exponents provided an additional check on the robustness of our conclusions. The indirect method was based on a neural network approach to estimate the deterministic skeleton of the dynamics[9,20]. This deterministic skeleton was used to calculate one Lyapunov exponent characterizing the dynamics of the entire food web.

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Author Contributions R.H. ran the experiment, counted the species and measured the nutrient concentrations. E.B., J.H., K.D.J., P.B. and S.P.E. performed the time series analysis. E.B., J.H., M.S. and S.P.E. wrote the manuscript. All authors discussed the results and commented on the manuscript.

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LETTERS

Removal of phospho-head groups of membrane lipids immobilizes voltage sensors of K^+ channels

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A fundamental question about the gating mechanism of voltage-activated K^+ (Kv) channels is how five positively charged voltage-sensing residues[1,2] in the fourth transmembrane segment are energetically stabilized, because they operate in a low-dielectric cell membrane. The simplest solution would be to pair them with negative charges[3]. However, too few negatively charged channel residues are positioned for such a role[4,5]. Recent studies suggest that some of the channel's positively charged residues are exposed to cell membrane phospholipids and interact with their head groups[5–9]. A key question nevertheless remains: is the phospho-head of membrane lipids necessary for the proper function of the voltage sensor itself? Here we show that a given type of Kv channel may interact with several species of phospholipid and that enzymatic removal of their phospho-head creates an insuperable energy barrier for the positively charged voltage sensor to move through the initial gating step(s), thus immobilizing it, and also raises the energy barrier for the downstream step(s).

Kv2.1 channels, expressed in *Xenopus* oocytes, interact with sphingomyelin[8] present mainly in the outer leaf of plasma membranes. To investigate the importance of phospho-head groups of membrane lipids in Kv-channel gating we employed bacterial sphingomyelinases C and D (SMases C and D)[10,11]. Both enzymes specifically hydrolyse sphingomyelin, but in different ways (Fig. 1a): SMase D removes only choline and leaves the lipid ceramide-1-phosphate behind in the membrane, whereas SMase C removes phosphocholine, leaving ceramide behind[12,13]. A comparison of the effects of these two enzymes on the channels will therefore help to reveal the functional significance of the phosphodiester group in voltage gating.

SMase D of *Corynebacterium pseudotuberculosis*[11] shifts the conductance–voltage (G–V) relation of Kv2.1 by about −30 mV (ref. 8) (Fig. 1d), allowing channels to be activated at a negative voltage at which they otherwise remain largely deactivated (Fig. 1c). The effect is maximal within 2 min and persists for at least 24 h (Fig. 1c, d; cells cannot regenerate sphingomyelin from ceramide-1-phosphate). It does not require direct exposure of channels to SMase D, because it also occurred in Kv2.1-expressing oocytes that had been treated with SMase D and then thoroughly washed before injection with Kv2.1 complementary RNA (cRNA; Fig. 1e). Additional exposure of such oocytes to SMase D, as expected, caused no further shift. SMase D therefore acts through its lipase activity rather than by direct binding to the channel. Our previous study[8] supports an electrostatic mechanism by which removal of the positively charged choline favours the activated state of the positively charged voltage sensors.

Unlike SMase D, SMase C removes the negatively charged phosphodiester group as well as choline. SMase C of *Bacillus anthracis*[14] (^{Ba}SMase C) decreases Kv2.1 current by about 90% (Fig. 2a–c) and the decrease is independent of voltage for both partial and complete enzymatic treatment (Fig. 2d). SMase C of *Staphylococcus aureus*[15]

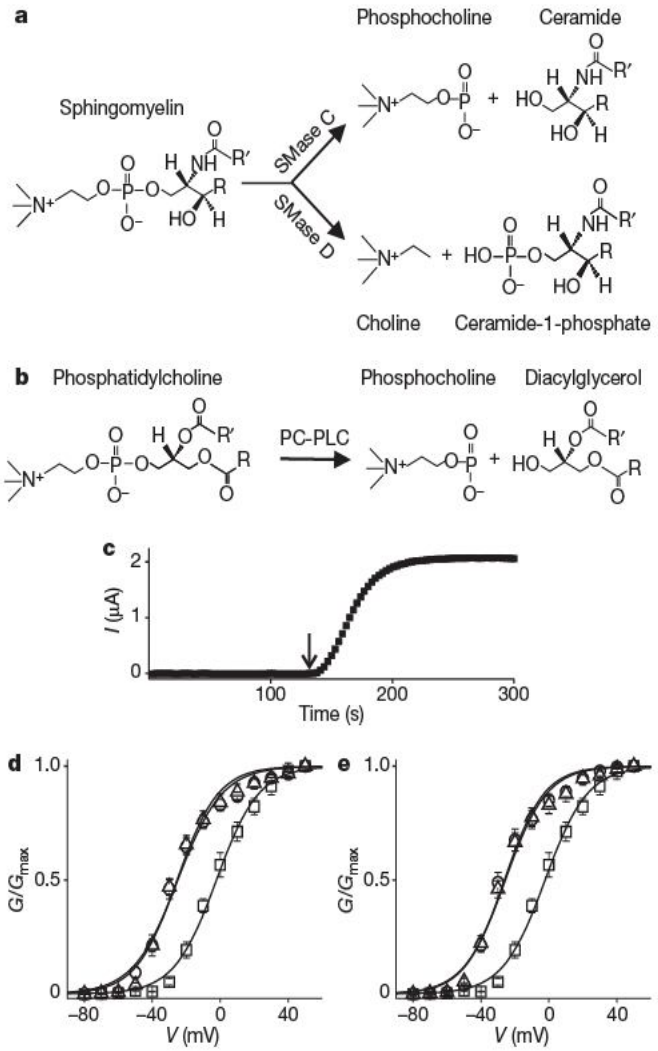

Figure 1 | Reaction schemes of lipid hydrolysis and the effect of SMase D on Kv2.1 channels. **a**, **b**, Hydrolysis of sphingomyelin by SMases C or D (**a**) and of phosphatidylcholine by PC-PLC (**b**). R and R′ represent acyl chains. **c**, Amplitude of Kv2.1 current, repeatedly elicited by stepping membrane voltage from −80 to −40 mV, where the arrow indicates the addition of recombinant SMase D to the bath. **d**, G–V relations obtained before treatment with SMase D (squares), and 30 min (circles) or 24 h (triangles) after exposure of oocytes to SMase D for 10 min. **e**, Oocytes were exposed to SMase D for 30 min and then washed with enzyme-free solution before being injected with Kv2.1 cRNA. Data were collected from each oocyte 18 h after the injection (circles), and again after an additional 15 min of treatment with SMase D (triangles). The control G–V relation was obtained without SMase D treatment (squares). All data in **d** and **e** are presented as means ± s.e.m. ($n = 5$), and the controls were pooled.

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(^{Sa}SMase C) caused a comparable level of inhibition but with faster kinetics, and only the kinetics—not the extent—of inhibition are dependent on enzyme concentration (Fig. 2b). SMase C therefore inhibits the channels by means of its enzymatic activity rather than by direct binding (more evidence below).

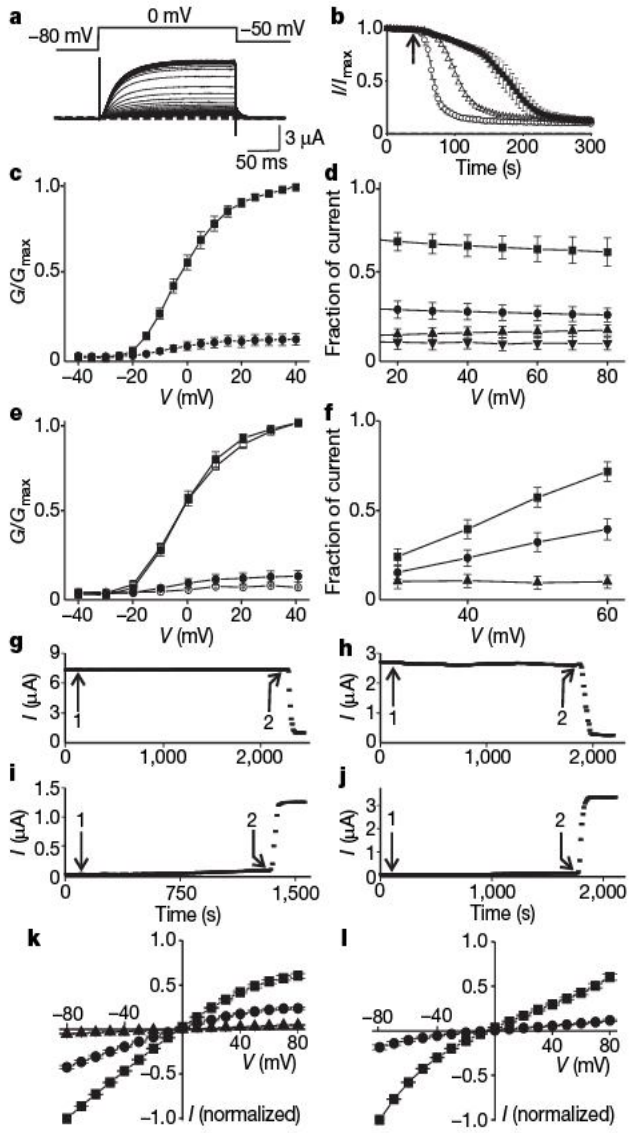

Figure 2 | Effects of SMase C on Kv2.1, Kir1.1 and a KcsA–Kir2.1 chimaera. **a**, Kv2.1 currents elicited at 5-s intervals with the voltage protocol shown, gradually declining after the addition of recombinant ^{Ba}SMase C to the bath. The dotted line indicates zero current level. **b**, Time course of current, where the arrow indicates the addition of 14 ng μl^{-1} (open circles, left) or 1.4 ng μl^{-1} (open triangles, middle) ^{Sa}SMase C, or 40 ng μl^{-1} ^{Ba}SMase C (filled squares, right). **c**, *G–V* curves of Kv2.1 before (squares) and after (circles) treatment with SMase C. **d**, Fraction of current remaining at various times after the addition of ^{Sa}SMase C, plotted against voltage: the top three data sets correspond to different levels of partial enzymatic treatment and the bottom set to complete treatment. The currents were normalized to those before the addition of SMase C at the corresponding voltages. **e**, *G–V* curves without (open symbols) or with (filled symbols) incubation for 2 h in a solution containing 5 mM MβCD, and before (squares) or after (circles) treatment with ^{Sa}SMase C. **f**, Fraction of current remaining after the addition of 3 μM hanatoxin (squares, top) and subsequent treatment with ^{Sa}SMase C (circles, middle), or after treatment with ^{Sa}SMase C alone (triangles, bottom). **g–j**, Kv2.1 currents plotted against time, where the arrows labelled 1 indicate the addition of 10 mM phosphocholine (**g**), 0.25 mg ml^{-1} ceramide (C_8) pre-dissolved in ethanol (**h**), 10 mM choline (**i**) or 0.25 mg ml^{-1} ceramide-1-phosphate (C_{12}) pre-dissolved in dodecane/methanol (1:49 by vol.) (**j**). SMase C (**g**, **h**) or SMase D (**i**, **j**) were used as positive controls (arrows labelled 2). Similar results were observed in at least three experiments for each case. **k**, *I–V* curves of Kir1.1 before (squares) and after (circles) treatment with SMase C, and after subsequent application of 0.5 μM pore-blocking tertiapin-Q (triangles). **l**, *I–V* curves of a KcsA–Kir2.1 chimaera before (squares) and after (circles) treatment with SMase C. All data in **c-f**, **k** and **l** are presented as means ± s.e.m. (n = 5–8).

Sphingomyelin and cholesterol may form membrane domains called 'lipid rafts'. We used the cholesterol-extracting agent methyl-β-cyclodextrin (MβCD)[16] to test whether the disruption of sphingomyelin–cholesterol interactions underlies the SMase C effect. Exposure of oocytes to 5 mM MβCD for 2 h (a common regimen) had little effect on the *G–V* curves obtained before and after SMase C treatment (Fig. 2e). The channel-inhibitory effect of SMase C therefore does not seem simply to reflect the disruption of sphingomyelin–cholesterol interactions.

Inhibition of Kv2.1 function by removal of the phospho-head of sphingomyelin is consistent with the observation that the voltage-gated (six-transmembrane) bacterial channel KvAP, unlike the non-voltage-gated (two-transmembrane) bacterial channel MthK, is non-functional when reconstituted in a non-phospholipid bilayer[9], an observation suggesting that voltage sensors, not the ion conduction pore, require phospholipids for proper functioning. Pursuing that reasoning, we tested the effect of SMase C on two other non-voltage-gated (two-transmembrane) K^+ channels: Kir1.1 and a KcsA pore with the Kir2.1 cytoplasmic termini attached[17]. Our finding that SMase C profoundly inhibits K^+ conduction in both channels (Fig. 2k, l) indicates that sphingomyelin head groups are in fact crucial for the proper function of these two-transmembrane channels that lack voltage sensors. More direct approaches are therefore needed to assess how SMase C inhibits voltage-gated Kv2.1 channels.

Looking for evidence that the observed inhibition of Kv2.1 by SMase C results from the removal of sphingomyelin phospho-heads around voltage sensors, we tested whether hanatoxin, which is known to bind to voltage sensors[18,19], prevents SMase C from reaching the lipids and thus mitigates its effect (kinetics of hanatoxin inhibition and recovery[18] are slow compared with the rate of inhibition caused by SMase C). Exposure to 3 μM hanatoxin inhibits Kv2.1 current by about 70% at 30 mV and by about 30% at 60 mV (Fig. 2f). (The extent of current reduction does not reflect the extent of hanatoxin binding; this is because a channel bound with hanatoxin can still be activated by a depolarization that is stronger than usual[19,20].) In the absence of hanatoxin, SMase C decreases current by 90% at all voltages, but in the presence of hanatoxin the observed decrease in current is much smaller (Fig. 2f). Hanatoxin binding therefore protects a large fraction of channels against SMase C, which supports the notion that SMase C inhibits the channels by removing sphingomyelin phospho-heads around voltage sensors.

We next examined whether SMase C affects gating current, a capacitive current arising from voltage sensor movement[21]. Unfortunately, measuring the gating current of Kv2.1 is technically challenging as a result of the lack of high-expression mutants that produce large gating currents but little ionic current. To circumvent this limitation, we employed the well-studied Shaker(-IR[22]) Kv channel for ionic current, and its non-conducting V478W mutant[23] for gating current measurements. SMase C treatment decreases both ionic and gating currents by about half (Fig. 3a–d). Such a proportional decrease could occur if about half of the channels interact with sphingomyelin in such a manner that removal of the negatively charged phosphate groups creates an insuperable energy barrier for the positive gating charges to move during early gating transitions, effectively precluding activation by experimentally accessible depolarizations. Alternatively, the proportional decrease might be a coincidence, peculiar to this particular Kv subtype, such that half of the gating charges in individual channels remain functional and these channels are therefore susceptible to partial activation. However, as shown below, we similarly observed an approximately proportional decrease in ionic and gating currents in Kv1.3 channels. Additionally, SMase C does not decrease the slope of the *Q–V* curve (Fig. 3f), a parameter related to the effective number of gating charges. It is thus more probable that in about half of the Shaker channels (more than half for Kv1.3 and Kv2.1), sphingomyelin

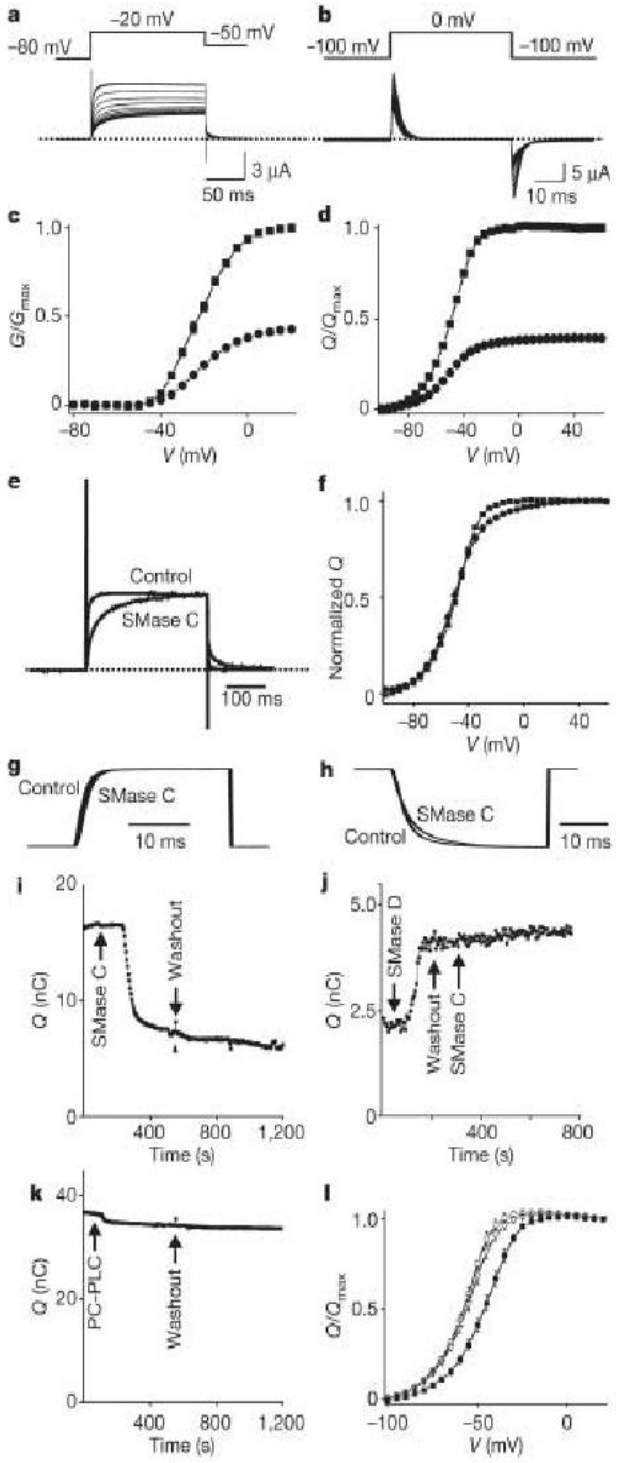

Figure 3 | Effect of SMase C and PC-PLC on ionic and gating currents of Shaker channels. **a**, **b**, Ionic currents of Shaker(-IR) (**a**) and gating currents of the V478W Shaker mutant (**b**) elicited with the protocols shown, gradually decreasing on addition of recombinant ^{Ba}SMase C. **c**, **d**, G–V (**c**) and Q_{on}–V (**d**) relations before (squares) or after (circles) treatment with SMase C. **e**, Trace of ionic current after treatment with SMase C, normalized in amplitude to that before treatment in **a**. **f**, Normalized Q–V curves before (squares) and after (circles) treatment with SMase C in **d**. **g**, **h**, Integrals of on (**g**) and off (**h**) gating charges (before or after treatment with SMase C) against time; all traces are normalized in amplitude. **i**–**k**, On-gating currents of Shaker's V478W mutant versus time (similar results were observed in at least three experiments for each case). Gating currents were collected by stepping the voltage from −100 mV to 0 mV (**i**, **k**) or −60 mV (**j**). The arrows indicate the addition and washout of SMase C (**i**), the addition and washout of SMase D and subsequent addition of SMase C (**j**), and the addition and washout of purified native PC-PLC (**k**). The current was on average decreased by 11.2 ± 2.8% (means ± s.e.m., $n = 3$) after the addition of PC-PLC. **l**, Q–V curves of control (filled squares) or after treatment with SMase D (open triangles) and subsequent treatment with SMase C (open circles). All data in **c**, **d**, **f** and **l** are presented as means ± s.e.m. ($n = 5$–11); the error bars in **c**, **d** and **f** are generally smaller than the symbols.

phospho-heads are essential in allowing voltage sensors to undergo early transitions involving the bulk of gating-charge movement. Phospholipids other than sphingomyelin presumably fulfil that role in the remaining channels. The two channel populations may exist in membrane domains that differ in their sphingomyelin content.

The above inference that the head groups of other phospholipids besides sphingomyelin enable early gating transitions in about half of Shaker channels expressed in *Xenopus* oocytes does not exclude the possibility that voltage sensors in these channels still interact with sphingomyelin, but in a manner that is important for late (downstream) transitions only. Rather, the very existence of channels whose early transitions do not require sphingomyelin permits investigation of the impact of sphingomyelin phospho-head groups on late transitions. We found that the kinetics of ionic currents that remain after SMase C treatment is markedly slowed (Fig. 3e), whereas that of the remaining gating currents is barely affected (Fig. 3g, h). Removal of phospho-head groups of sphingomyelin must therefore also increase (but not insuperably) the energy barrier for one or more gating transitions downstream of the bulk of the gating charge movement. This modest increase in the transition energy for one or more late rate-limiting steps is consistent with the occurrence of only 5–10% of total gating charge movement late in the gating sequence[24,25]. The resulting small change in the shallow part of the normalized Q–V curve at more depolarized potentials (Fig. 3f) is reminiscent of an S4 mutant (L370V) whose late transition is altered[24].

Consistent with our early conclusion that SMase C acts by means of its lipase activity was our observation that gating current did not recover after washout of SMase C (Fig. 3i). For further confirmation, we exploited the fact that SMase C acts only on sphingomyelin, not on ceramide-1-phosphate. As expected, conversion of sphingomyelin to ceramide-1-phosphate with SMase D shifts the activation curve to the left (Fig. 3l); that is, it allows the mobilization of gating charges at more negative voltages (Fig. 3j). Indeed, pretreatment with SMase D prevents subsequently added SMase C from causing current inhibition (Fig. 3j, l). The differing effects of SMases C and D indicate that the lipid product of one or both of the enzymes continues to interact with the channel protein for the duration of the experiment. The persistence probably reflects strong channel–lipid interactions and/or the confinement of relevant lipid molecules to microdomains. Consistent with this view was our observation that channel current was not significantly affected by the acute addition of products of sphingomyelin hydrolysis catalysed by either enzyme (Fig. 2g–j).

To rule out the possibility that the observed effect of SMase C results from non-specific hydrolysis of the predominant outer-leaf phospholipid phosphatidylcholine (PC), we tested a PC-specific phospholipase (PC-PLC) (Fig. 1b) from *Bacillus cereus* directly against the channels, and found only modest (11.2 ± 2.8%) gating current depression (Fig. 3k), in contrast with about 50% for SMase C. SMase C therefore does not cause channel inhibition by hydrolysing PC. The small inhibition caused by PC-PLC is, again, consistent with the interaction of some channels with phospholipids other than sphingomyelin.

SMase C was originally termed β-haemolysin after its haemolytic effect *in vitro*[10], yet its role in pathogenesis remains largely unknown. The fact that inhibition of Kv1.3 in lymphocytes[26,27] is immunosuppressive[28] motivated us to test ^{Sa}SMase C against human Kv1.3. We found that ^{Sa}SMase C eliminates well over half of the ionic current of Kv1.3 and of the gating current of its non-conducting W384F mutant[29] (Fig. 4), in an approximately proportional manner as with Shaker channels. This finding raises the intriguing possibility that the SMase C action against Kv1.3 helps *S. aureus* to neutralize host defences.

Thus, the voltage sensor of Kv channels may interact strongly with multiple molecules of several kinds of phospholipid. In the present experiments on *Xenopus* oocytes, sphingomyelin seems to be preferred over PC (these lipids differ in hydrogen-bonding

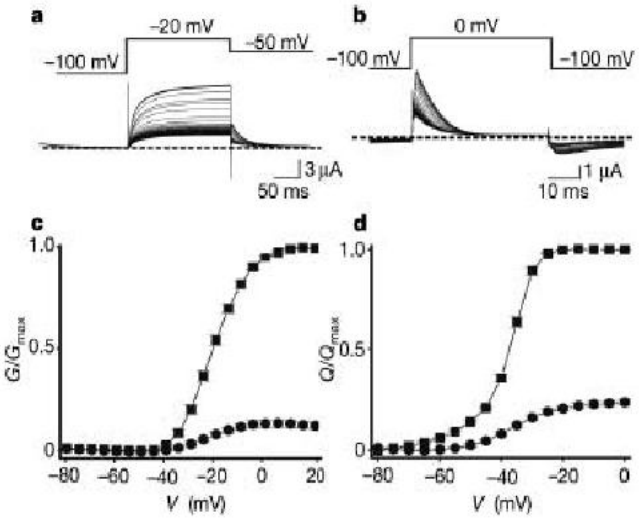

Figure 4 | Inhibition of Kv1.3 by SMase C. **a**, **b**, Ionic currents of wild-type Kv1.3 (**a**) and gating currents of the W384F mutant (**b**) elicited at 5-s intervals with the voltage protocol shown, gradually declining after the addition of recombinant ^{Sa}SMase C (the off-gating current is largely immobilized). **c**, **d**, G–V (**c**) and Q_{on}–V (**d**) relations before (squares) and after (circles) treatment with ^{Sa}SMase C. The data are presented as means ± s.e.m. (n = 9–11).

characteristics). The apparent preference probably reflects both inherent channel–lipid affinity and/or relative abundance in the channels' lipid microenvironments. Some interactions between voltage sensors and phospholipids are important for the early gating transitions, and others are important for late transitions. These findings strongly support the hypothesis that phospho-head groups of membrane lipids, together with certain acidic channel residues[4,5], provide the necessary counter-charges for the positively charged voltage-sensing residues during individual steps of the voltage-sensor movement. This charge-neutralizing action lowers the free energy of the overall gating process as well as the energy barrier for individual transitions, so that a modest voltage can drive the charged sensors through a series of conformational steps in a low-dielectric cell membrane to open the channel gate.

METHODS SUMMARY

Channel currents were recorded under two-electrode voltage clamping from oocytes injected with cRNA encoding relevant channels. The bath solution contained (in mM): 95/5 NaCl/KCl (or 80/20 for tail current measurements), 0.3 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES, pH 7.6. Chemical reagent and lipase stock solutions (2 μl) were added manually to a 100-μl recording chamber. Unless specified otherwise, the final concentrations of recombinant[30] ^{Ba}SMase C, ^{Sa}SMase C, SMase D and native PC-PLC were 40, 14, 4 and 50 ng μl^{-1}, respectively.

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LETTERS

A modular switch for spatial Ca^{2+} selectivity in the calmodulin regulation of Ca_V channels

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Ca^{2+}/calmodulin-dependent regulation of voltage-gated Ca_V1–2 Ca^{2+} channels shows extraordinary modes of spatial Ca^{2+} decoding and channel modulation[1–6], vital for many biological functions[6–9]. A single calmodulin (CaM) molecule associates constitutively with the channel's carboxy-terminal tail[3,10–13], and Ca^{2+} binding to the C-terminal and N-terminal lobes of CaM can each induce distinct channel regulations[2,14]. As expected from close channel proximity, the C-lobe responds to the roughly 100-μM Ca^{2+} pulses driven by the associated channel[15,16], a behaviour defined as 'local Ca^{2+} selectivity'. Conversely, all previous observations have indicated that the N-lobe somehow senses the far weaker signals from distant Ca^{2+} sources[2,3,17,18]. This 'global Ca^{2+} selectivity' satisfies a general signalling requirement, enabling a resident molecule to remotely sense cellular Ca^{2+} activity, which would otherwise be overshadowed by Ca^{2+} entry through the host channel[5,6]. Here we show that the spatial Ca^{2+} selectivity of N-lobe CaM regulation is not invariably global but can be switched by a novel Ca^{2+}/CaM-binding site within the amino terminus of channels (NSCaTE, for N-terminal spatial Ca^{2+} transforming element). Native $Ca_V2.2$ channels lack this element and show N-lobe regulation with a global selectivity. On the introduction of NSCaTE into these channels, spatial Ca^{2+} selectivity transforms from a global to local profile. Given this effect, we examined $Ca_V1.2$/$Ca_V1.3$ channels, which naturally contain NSCaTE, and found that their N-lobe selectivity is indeed local. Disruption of this element produces a global selectivity, confirming the native function of NSCaTE. Thus, differences in spatial selectivity between advanced Ca_V1 and Ca_V2 channel isoforms are explained by the presence or absence of NSCaTE. Beyond functional effects, the position of NSCaTE on the channel's amino terminus indicates that CaM can bridge the amino terminus and carboxy terminus of channels. Finally, the modularity of NSCaTE offers practical means for understanding the basis of global Ca^{2+} selectivity[19].

Figure 1a, b shows a prototypic example of global Ca^{2+} regulation induced by Ca^{2+} binding to the N-lobe of CaM, here driving Ca^{2+}-dependent inactivation (CDI) of $Ca_V2.2$ channels[3]. On channel activation by a depolarizing voltage step (Fig. 1a), this CDI manifests itself as an accelerated decay of Ca^{2+} (middle, red trace) against Ba^{2+} current (black trace). The baseline decay in Ba^{2+}, which binds poorly to CaM[2], reflects a separate voltage-dependent inactivation process[7]. In population data (Fig. 1a, right), the fraction of peak Ca^{2+} current remaining after a 300-ms depolarization, r_{300}, is a U-shaped function of voltage (red circles), providing a hallmark of CDI[20]. The corresponding Ba^{2+} relation (Fig. 1a, black circles) shows a weak monotonic decline, and the difference between Ca^{2+} and Ba^{2+} relations (f_{300}) quantifies pure CDI[20]. In accord with a so-far invariant rule[3,6], N-lobe regulation of Ca^{2+} channels is selective for a global Ca^{2+} increase arising from spatially distant sources. Such global crosstalk is permitted under modest intracellular Ca^{2+} buffering[18] that approximates physiological conditions (Fig. 1a, left, shading with 0.5 mM EGTA). Thus, strong buffering that localizes Ca^{2+} to channel nanodomains[15,16] (Fig. 1b, left, small hemisphere in 10 mM BAPTA) almost eliminates CDI (Fig. 1b, middle and right).

Previous splice variations, mutations and chimaeras have at best altered the strength of such CDI[2,3,21], making global Ca^{2+} preference seem immutable. Here, however, when the N terminus of $Ca_V1.2$ channels (NT_C) was substituted into $Ca_V2.2$, the resulting 'cBBBBb' chimaera showed strong N-lobe CDI in high buffering (Fig. 1c and Supplementary Information). Introducing NT_C into $Ca_V2.1$ produced an analogous result (data not shown), thus generalizing the effect across the Ca_V2 family. This conversion to local Ca^{2+} selectivity is unprecedented, especially given the preponderance of known structural determinants for CDI on the carboxy termini of channels[22].

To explore the basis of this effect, we undertook progressive amino-terminal deletions from the NT_C segment within the cBBBBb chimaera. Deletion of the first 81 residues (Δ82cBBBBb) completely spared CDI in high buffering (Fig. 1d, top left, where the grey trace shows the full-length NT_C profile). By contrast, removing just one more residue (82W) significantly decreased CDI (Fig. 1d, top middle), and additional deletion further suppressed CDI (Fig. 1d, top right). Explicit correlation of CDI strength with NT_C deletion corroborated these trends (Fig. 1d, bottom, left axis, circles), localizing the impact to a short contiguous region (yellow highlight).

What could the essential mechanistic ingredient of this locus be? We reasoned that this segment might orchestrate special molecular interactions with cytoplasmic channel loops or modulatory ligands, and thus probed for such associations with the use of a live-cell fluorescence resonance energy transfer (FRET) two-hybrid assay[13]. Using this approach, with enhanced yellow fluorescent protein fused to CaM (EYFP–CaM), and enhanced cyan fluorescent protein (ECFP) to various portions of NT_C or the $Ca_V2.2$ amino terminus (NT_B), we found a unique ability of NT_C to bind Ca^{2+}/CaM within the intracellular milieu of HEK-293 cells (Fig. 1e). To start with, NT_B failed to display FRET interaction with CaM, either in the Ca^{2+}-free or the Ca^{2+}-bound state (Fig. 1e, middle column, topmost bars). Specifically, the optical parameter FRET ratio (FR) was about 1, signifying the absence of FRET[13]. By contrast, NT_C clearly interacted with Ca^{2+}/CaM (FR ≈ 2), but not apoCaM (Ca^{2+}-free CaM). Trisection of NT_C localized Ca^{2+}/CaM binding to the middle third (Fig. 1e, middle column, residues 67–131), and further deletions identified a sharp decrease in FRET on removal of W82 (Supplementary Information). Because FR depends on the fractional binding between interacting partners, cell-to-cell variation in expression permitted the resolution of binding curves, each specifying a relative

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dissociation constant $K_{d,EFF}$ (ref. 13) (Fig. 1e, right column, arrow). Alignments of these NT_C fragments and their $K_{d,EFF}$ values (Fig. 1d, squares, and Supplementary Information) localized a CaM-binding segment that coincided well with the critical CDI segment (Fig. 1d, yellow highlight). In particular, a W82A point mutation suppressed the interaction of Ca^{2+}/CaM with NT_C (Fig. 1f), fitting with the decrease in CDI of the corresponding channel truncation (Fig. 1d, top row, middle). Thus, the transformation of spatial Ca^{2+} selectivity is correlated with a previously unrecognized Ca^{2+}/CaM interaction site within NT_C.

This connection was deepened in two ways (Fig. 2). First, the *in situ* FRET approach was confirmed by *in vitro* assays, pairing purified Ca^{2+}/CaM with a synthetic peptide (SWQAAIDAARQAKLMGSA) spanning the key NT_C binding region. Without peptides, CaM migrated rapidly under non-denaturing PAGE[2] (Fig. 2a, left, lane 1, open arrowhead). When fully complexed with the IQ domain peptide of $Ca_V2.1$ (IQ_A), CaM showed a known slowing of mobility[2] (Fig. 2a, left, lane 2, filled arrowhead). Increasing $NT_{C\text{-peptide}}$ produced progressive conversion to the faster mobility species (Fig. 2a, left, lanes 3–8), indicating competitive binding to CaM. By contrast, a mutant W82A peptide ($NT_{C\text{-peptide-(W82A)}}$; Fig. 2a, right) was unable to bind CaM. More quantitatively, $NT_{C\text{-peptide}}$ depressed the emission spectrum of dansylated CaM (Fig. 2b, inset), allowing the resolution of a 1:1 binding curve with a K_d of 1.2 μM (Fig. 2b, filled symbols). $NT_{C\text{-peptide-(W82A)}}$ showed no such interaction (Fig. 2b, open symbols). These *in vitro* assays established direct Ca^{2+}/CaM binding to the NT_C core region.

Second, further in-depth mapping refined the correlation between spatial Ca^{2+} selectivity and CaM binding to NT_C. Alanine point mutations were introduced into the NT_C domain of cBBBBb channels, targeting key sites inferred from a preliminary report[19]. These manipulations decreased CDI (in high buffering) with the rank order W82A > I86A > R90A (Fig. 2c). Reassuringly, these changes in CDI (f_{300}) correlated closely with the relative dissociation constant ($K_{d,EFF}$) for CaM interaction with corresponding mutant NT_C peptides, as determined by FRET (Fig. 2d and Supplementary Information). Given the strong function and affiliation with CaM binding to the core NT_C locus, we named this element NSCaTE (Fig. 2c, top).

Although this module impacts chimaeric channels, does NSCaTE function within naturally occurring channels? Alignments of various Ca_V1–2 Ca^{2+} channels (Fig. 3a) revealed that NSCaTE is present not only in $Ca_V1.2$ (α_{1C}), but also $Ca_V1.3$ (α_{1D}). Our attention was initially drawn to NSCaTE in $Ca_V1.3$ because these channels show two distinct and robust forms of CDI, each selectively triggered by Ca^{2+} binding to a different lobe of CaM[14]. As a preliminary test for the functionality of the $Ca_V1.3$ NSCaTE, we checked for Ca^{2+}/CaM interaction (Fig. 3b). FRET assays showed selective interaction of Ca^{2+}/CaM with the midsection of the $Ca_V1.3$ amino terminus (Fig. 3b; NT_D-(35–94)), yielding a $K_{d,EFF}$ on a par with those of NT_C analogues (Fig. 1e). Consistent with W82A effects in NT_C (Fig. 1f), mutation of the analogous tryptophan in NT_D (NT_D-W44A-(35–94)) eliminated Ca^{2+}/CaM interaction (Fig. 3b and Supplementary Information). Substituting NT_D into $Ca_V2.2$ (yielding the dBBBBb chimaera) also produced strong CDI in high buffering (Fig. 3c and Supplementary Information), confirming the transforming capacity of the $Ca_V1.3$ NSCaTE.

We next examined whether the intrinsic NSCaTE imparts local selectivity to the native $Ca_V1.3$, contrary to the current dogma that the N-lobe invariably senses global Ca^{2+}. In $Ca_V1.3$, the N-lobe form of CDI can be isolated by simultaneously expressing a mutant CaM

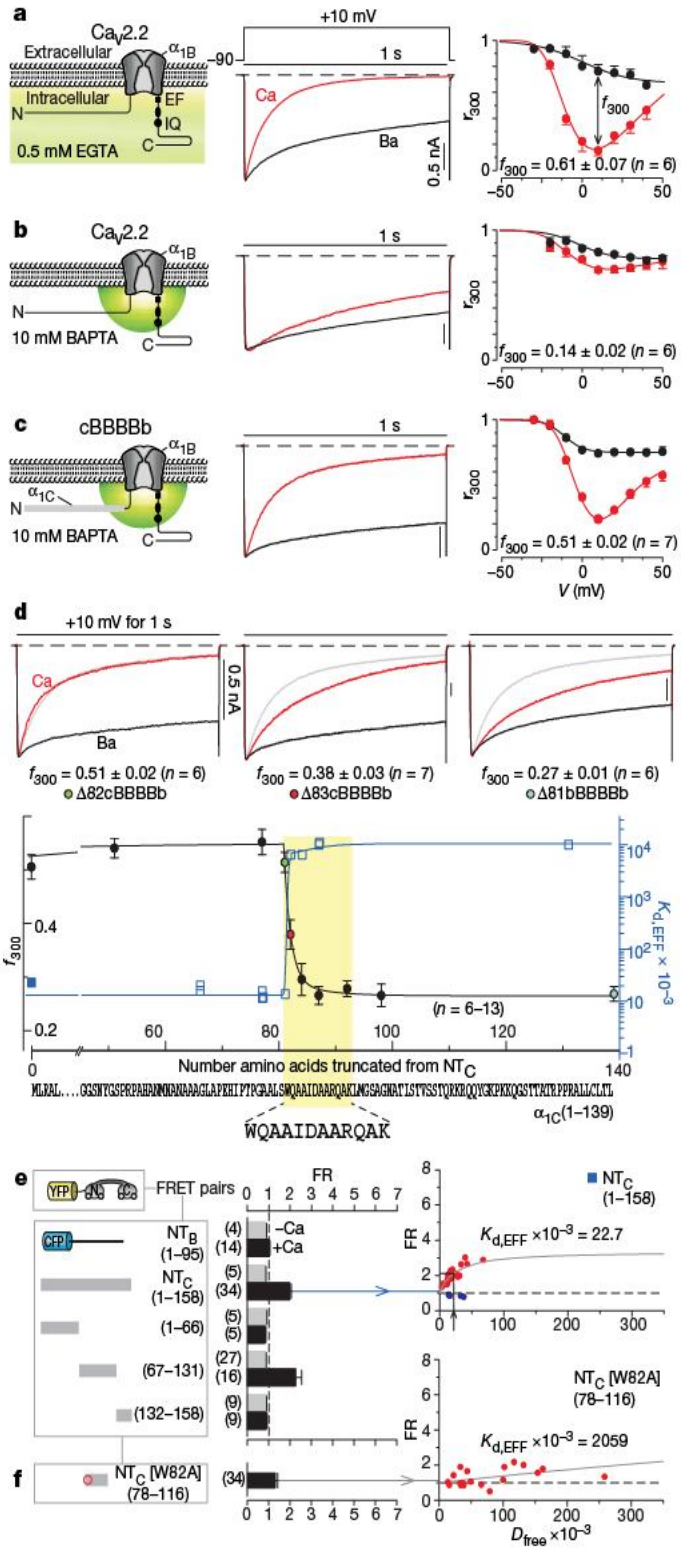

Figure 1 | Transformation of spatial Ca^{2+} selectivity in $Ca_V2.2$ channels. **a**, $Ca_V2.2$ CDI, low buffering. Left: diagram of global Ca^{2+} elevation. Middle: example traces in Ca^{2+} (red) and Ba^{2+} (black). Throughout, the current bar refers to the Ca^{2+} trace, and the Ba^{2+} trace is normalized to the Ca^{2+} peak. Right: average CDI; f_{300} at 10 mV; results are means ± s.e.m. throughout; cell numbers are shown in parentheses. **b**, $Ca_V2.2$ CDI, high buffering. Left: diagram of local Ca^{2+} signalling. **c**, CDI of cBBBBb. **d**, Localizing spatial Ca^{2+} transforming element (yellow highlight) within NT_C. Top: example currents for cBBBBb with N-terminal deletions, (Δ82cBBBBb, removal of first 81 residues of cBBBBb; Δ81bBBBBb, removal of entire amino terminus of $Ca_V2.2$). Middle: f_{300} (high buffering) versus residues deleted from cBBBBb; left axis and circles with colours correspond to examples above. $K_{d,EFF}$ is also plotted on same abscissa; blue right axis and blue squares, with filled symbol corresponding to **e**. Bottom: localized sequence for both CDI transformation and Ca^{2+}/CaM binding within NT_C (yellow highlight). **e**, **f**, FRET for YFP–CaM versus channel amino-terminal segments tagged with CFP. Left: construct schematics. Middle: FRET ratios (FR). Right: example binding curves, with red symbols for Ca^{2+}/CaM, and blue symbols for apoCaM (arrow in **e** shows $K_{d,EFF}$). D_{free}, relative concentration of CFP-tagged molecules[13]. **f**, Abolition of FRET between YFP–CaM and a CFP-tagged channel amino-terminal segment with a W82A mutation.

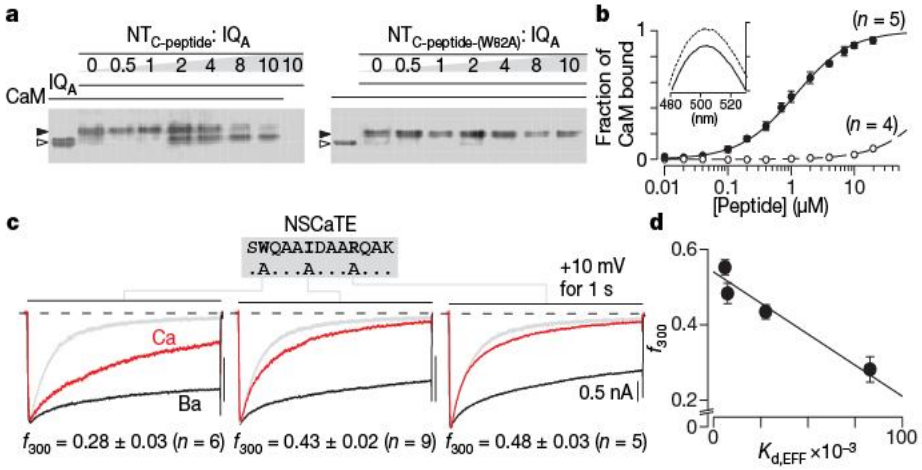

Figure 2 | Direct CaM binding and mapping of key NSCaTE residues. **a**, Competitive gel-mobility shift assay, confirming Ca^{2+}/CaM interaction with $NT_{C\text{-}peptide}$ (left), not mutant $NT_{C\text{-}peptide\text{-}(W82A)}$ (right). **b**, Dansylated Ca^{2+}/CaM spectrofluorimetry. Binding with $NT_{C\text{-}peptide}$ (filled circles), not with mutant $NT_{C\text{-}peptide\text{-}(W82A)}$ (open circles), both plotted as means ± s.e.m. Inset: $NT_{C\text{-}peptide}$ decreases the emission spectrum. **c**, CDI in high buffering, for Δ78cBBBBb channels with point mutations as labelled. Format as in Fig. 1a; the grey trace shows the baseline Δ78cBBBBb profile. **d**, Close correlation of CDI in high buffering with NT_C module affinity for Ca^{2+}/CaM_{34} (Supplementary Information).

(CaM_{34}) in which Ca^{2+} binding is restricted to the N-lobe[2,20]. So far, such CDI has been studied only under modest Ca^{2+} buffering[14]. Here, even under high buffering, N-lobe CDI was pronounced (Fig. 3d, top row). More tellingly, a W44A mutation within $Ca_V1.3$ almost eliminated this CDI (Fig. 3d, middle row), as did deletion of this region (Supplementary Information). To exclude indiscriminate disruption of CDI, we confirmed that CDI resurfaced under modest buffering permissive of global signalling (Fig. 3d, bottom row, and Supplementary Information). CDI was entirely CaM-mediated under decreased buffering[14] (Supplementary Information), and C-lobe CDI was unaffected by similar mutations[19]. Hence, the native $Ca_V1.3$ NSCaTE endows N-lobe CDI with a local selectivity, and decreasing Ca^{2+}/CaM binding to NSCaTE switches this selectivity towards a global profile.

Returning to native $Ca_V1.2$, coarse identification of Ca^{2+}/CaM binding to the N terminus of these channels has been reported, but the functional consequences have been unclear[23]. In particular, the CaM-mediated CDI of these channels has been attributed only to the C-lobe of CaM[7,20]; apparently absent has been an N-lobe form of CDI, the target of NSCaTE effects. Here, however, on simultaneous transfection of $Ca_V1.2$ with CaM_{34}, to isolate a potential N-lobe component, small but unmistakable CDI was seen during prolonged 1-s depolarization, even in high buffering (Fig. 3e, top row). In addition, a W82A mutation abolished this CDI (Fig. 3e, middle row), but

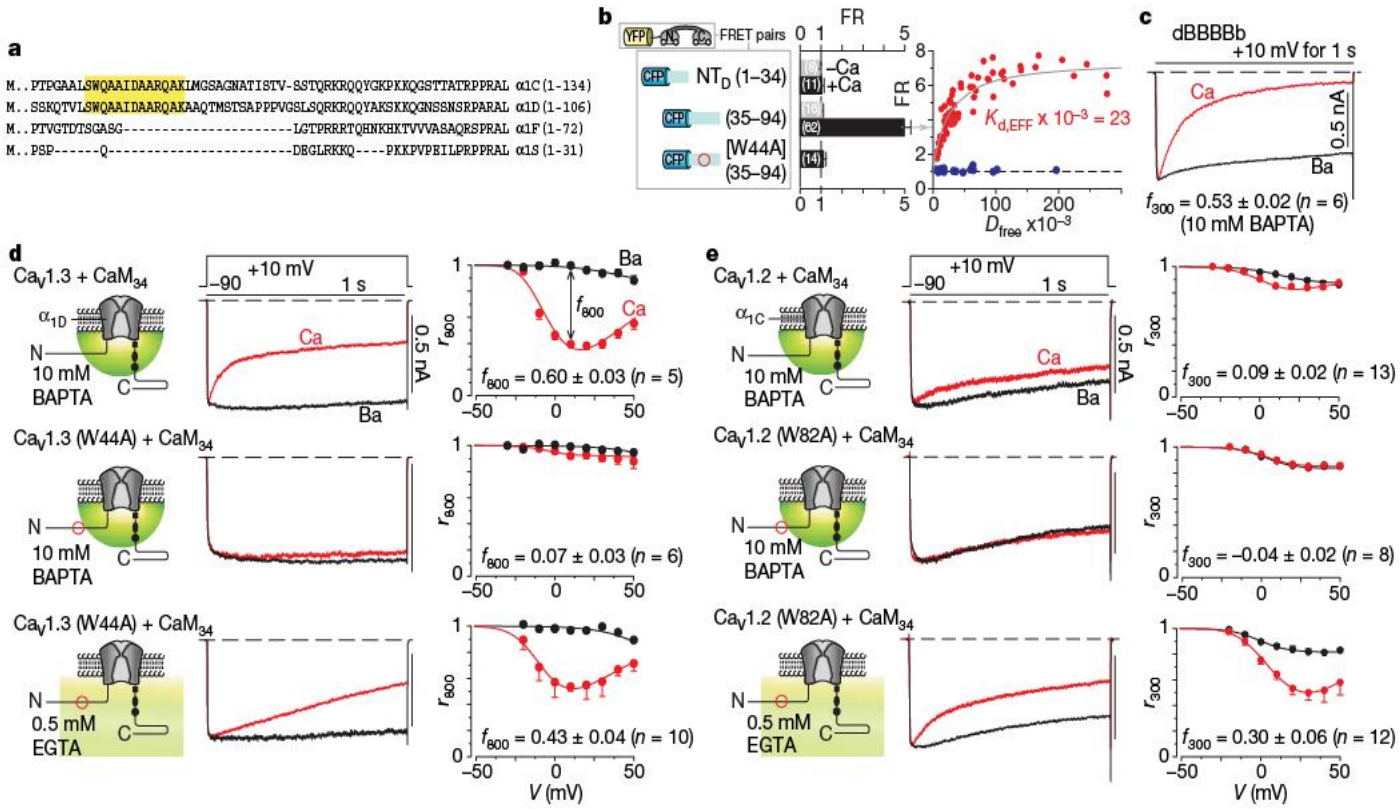

Figure 3 | NSCaTE transforms spatial Ca^{2+} selectivity in native Ca_V1 channels. **a**, α_1 alignment, Ca_V1 channels. **b**, FRET assays. Format as in Fig. 1e. **c**, CDI of dBBBBb in high buffering. **d**, Effect of native NSCaTE on N-lobe CDI of $Ca_V1.3$. Top: CDI persists in high buffering. Middle: CDI is eliminated by W44A mutation. Bottom: CDI reappears in W44A mutant under low buffering. Format as in Fig. 1a, except for 800-ms metrics. **e**, Native NSCaTE effects in $Ca_V1.2$. Format as in **d**, with 300-ms metrics.

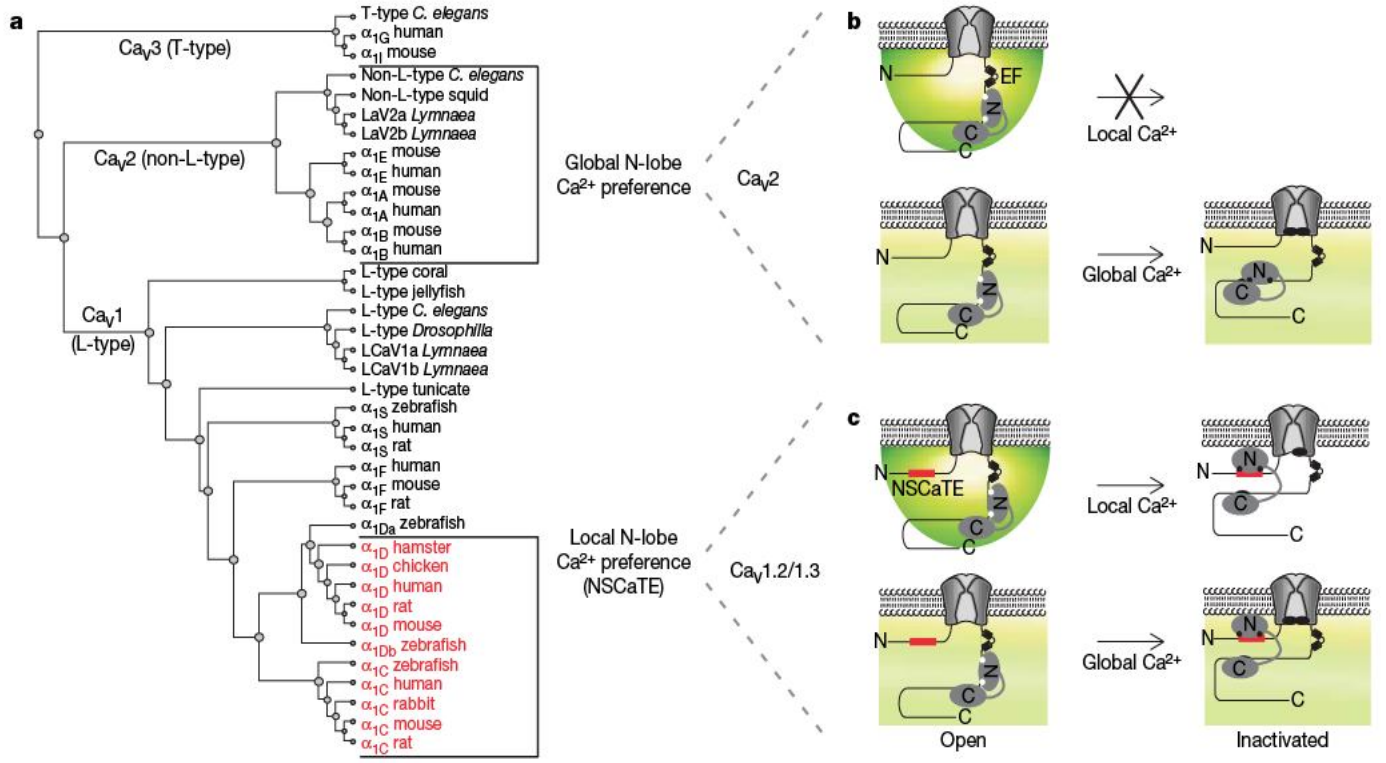

Figure 4 | Functional and structural properties for NSCaTE switching of spatial Ca^{2+} selectivity. **a**, Ca$_V$1–2 dendrogram, based on α_1 subunits (Supplementary Information). Red, NSCaTE-containing channels. **b**, Diagram of N-lobe CDI in Ca$_V$2 channels. Absence of NSCaTE yields global Ca^{2+} selectivity. Black circles on CaM, Ca^{2+} ions. **c**, N-lobe CDI in Ca$_V$1 channels that contain NSCaTE (red) show local Ca^{2+} selectivity. CaM bridges the amino and carboxy termini of inactivated channels (right).

spared it under global Ca^{2+} signalling (bottom row). A more drastic deletion of the native NSCaTE produced an identical effect (Supplementary Information). Again, the globally signalled CDI remained entirely CaM dependent (Supplementary Information). Hence, NSCaTE functions similarly in native Ca$_V$1.2 and Ca$_V$1.3; the weaker N-lobe CDI of Ca$_V$1.2 is expected from its lower open probability[19,24]. These results generalize the lessons of chimaeric channels to the operation of native channels and constitute the first examples in which the N-lobe of CaM shows local Ca^{2+} selectivity.

In all, the traditionally global spatial Ca^{2+} selectivity of channel regulation by the N-lobe[3,6] is not invariant but can be transformed towards a local selectivity by NSCaTE, a Ca^{2+}/CaM-binding site unrecognized by current motif-detection algorithms[25]. Four perspectives emerge. First, the presence of NSCaTE in Ca$_V$1.2 and Ca$_V$1.3—along with its absence from Ca$_V$2 channels—underlies the contrasting spatial Ca^{2+} preferences of N-lobe CDI between these two channel clades (Fig. 4a). Previously, N-lobe regulation had been viewed as always having a global Ca^{2+} selectivity[3,6], which is true for Ca$_V$2 channels (Fig. 4b). We now recognize that the analogous N-lobe CDI of Ca$_V$1.2/Ca$_V$1.3 channels[14] exhibits local selectivity, owing to an intrinsic NSCaTE (Fig. 4c). Second, NSCaTE promises vital adjustments of spatial selectivity in various biological contexts. NSCaTE would customize Ca$_V$1.2/1.3 regulation for local Ca^{2+} signalling in cardiac dyads[4,7], and for neuronal Ca^{2+} entry whose precise regulation is essential for long-term synaptic plasticity[26,27]. A naturally occurring Ca$_V$1.2 splice variant features a premature stop codon just after the channel's amino terminus[21]. The predicted protein product contains NSCaTE and may exert a dominant-negative effect. Although expressing NT$_C$ and Ca$_V$1.3 together left CDI unchanged in HEK-293 cells (data not shown), additional mechanisms may exist in native tissues to render this splice product active. Third, unlike most functional motifs, which are pervasive from bacteria to advanced mammals[25], NSCaTE is found only in a subset of Ca$_V$1 channels from advanced species (Fig. 4a, red) and in multiple bacterial proteins (Supplementary Information). A prokaryotic NSCaTE from *Xanthomonas* may in fact bind endogenous CaM-like molecules (Supplementary Information), hinting that channel and bacterial motifs share a common heritage. Last, NSCaTE furnishes valuable structural and mechanistic insights. For structure, we deduce that CaM can bridge the carboxy and amino termini of Ca$_V$1.2/Ca$_V$1.3 channels (Fig. 4c, right), as follows. This study establishes Ca^{2+}-bound N-lobe interaction with NSCaTE on the channel's amino terminus. By contrast, previous reports emphasized that regulation by the C-lobe involves its Ca^{2+}-bound and Ca^{2+}-free interactions with the channel's carboxy terminus[12,20,28]. Moreover, a single resident CaM orchestrates both C-lobe and N-lobe regulation[10,11], where both coexist in Ca$_V$1.2/Ca$_V$1.3 channels[14,20]. Finally, the channel's amino and carboxy termini are in close proximity, and move with channel gating[29]. This bridging therefore seems probable, expanding a theme where the lobes of CaM crosslink separate parts of a molecule[30]. As for mechanism, the modularity of NSCaTE indicates that spatial Ca^{2+} preference is not hopelessly intertwined within holochannel structure. Manipulating this compact element promises to provide insights into the elusive mechanism underlying spatial Ca^{2+} selectivity[6,19].

METHODS SUMMARY

Molecular biology. Channel chimaeras, mutations and CFP/YFP-FRET constructs were prepared with standard molecular biology techniques.

Electrophysiology. Transient transfection of HEK-293 cells with recombinant channels, and whole-cell current recordings, were performed mostly as described previously[2].

CaM binding assays. FRET two-hybrid experiments were performed in HEK-293 cells as described[13]. Gel mobility-shift assays were conducted in accordance with protocols similar to those in our previous work[2,20]. Spectrofluorimetric determinations of dansyl-CaM interaction were conducted as outlined previously[2,3,20].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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LETTERS

Co-option of a default secretory pathway for plant immune responses

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Cell-autonomous immunity is widespread in plant–fungus interactions and terminates fungal pathogenesis either at the cell surface or after pathogen entry. Although post-invasive resistance responses typically coincide with a self-contained cell death of plant cells undergoing attack by parasites, these cells survive pre-invasive defence. Mutational analysis in *Arabidopsis* identified *PEN1* syntaxin as one component of two pre-invasive resistance pathways against ascomycete powdery mildew fungi[1–3]. Here we show that plasma-membrane-resident PEN1 promiscuously forms SDS-resistant soluble *N*-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complexes together with the SNAP33 adaptor and a subset of vesicle-associated membrane proteins (VAMPs). PEN1-dependent disease resistance acts *in vivo* mainly through two functionally redundant VAMP72 subfamily members, VAMP721 and VAMP722. Unexpectedly, the same two VAMP proteins also operate redundantly in a default secretory pathway, suggesting dual functions in separate biological processes owing to evolutionary co-option of the default pathway for plant immunity. The disease resistance function of the secretory PEN1–SNAP33–VAMP721/722 complex and the pathogen-induced subcellular dynamics of its components are mechanistically reminiscent of immunological synapse formation in vertebrates, enabling execution of immune responses through focal secretion.

Arabidopsis is immune to non-adapted powdery mildew fungi such as *Blumeria graminis* and *Erysiphe pisi*, which in nature colonize grass and pea species, respectively. This non-host resistance requires both pre- and post-invasive immune responses, which are under separate genetic control[2]. The former response engages PEN1 syntaxin, peroxisomal PEN2 β-glycosyl hydrolase and the plasma-membrane-resident PEN3 ABC transporter[1–3]. PEN2 and PEN3 act in the same pathway and are implicated in the cytoplasmic synthesis and transport of small antimicrobial compounds across the plasma membrane at attempted fungal entry sites, respectively[2,3]. PEN1 syntaxin acts in a second pathway and could, by analogy to known syntaxin functions in yeast and animals, either participate in vesicle fusion processes[4] or modulate ion-channel activity through interactions with plasma-membrane-resident ion channels[5]. Genetic studies defy mechanistic interpretations but suggest direct or indirect PEN1 repressor activity in defence responses that are dependent on salicylic acid, as well as an overlapping function with the closely related syntaxin of plant 122 (SYP122)[6].

Compared with largely resistant *PEN1* wild type and severely defence-compromised *pen1*-1 null mutants, plants containing the *pen1*-3 allele allow intermediate *B. graminis* entry rates, indicating residual PEN1-3 resistance activity (Supplementary Fig. 1a). In the deduced PEN1-3 protein, a glycine residue is substituted by a glutamate in the SNARE domain[1] (Supplementary Fig. 1b). Because this mutation affects a hydrophobic residue that is thought to stabilize interactions with other SNARE proteins in hetero-oligomeric SNARE complexes[7] and is conserved in the SYP12 subfamily[8], we hypothesized that PEN1-3 might be impaired in SNARE complex formation. Immunoblot analysis of leaf protein samples from wild-type, *pen1*-1 and *pen1*-3 plants with a PEN1 antiserum showed that monomeric PEN1-3 and wild-type PEN1 levels are indistinguishable and that PEN1-1 is undetectable (Fig. 1a), thus demonstrating that the amino-acid exchange in PEN1-3 does not affect protein stability. Notably, this analysis revealed small amounts of an SDS-resistant and heat-labile PEN1-containing complex in wild-type plants whose abundance increased upon fungal inoculation (Fig. 1a). This complex migrated at about 150 kilodaltons (kDa) and was undetectable in *pen1*-3 plants (see over-exposed immunoblot; Fig. 1a). Because SDS resistance and heat sensitivity are typical features of exocytic ternary SNARE complexes in yeast and animals[9], we concluded that *in planta* PEN1-3 either has a specific defect in SNARE complex formation or that PEN1-3 complex is unstable.

Binary target membrane (t)-SNARE complexes are typically formed by a syntaxin and SNAP25 at the plasma membrane during exocytosis[4]. Hence, we examined the expression of all three *Arabidopsis* SNAP25 homologues, *SNAP29*, *SNAP30* and *SNAP33*, in leaves, the target tissue of powdery mildew fungi, by reverse transcriptase–polymerase chain reaction (RT–PCR) and detected exclusively SNAP33 transcripts (Supplementary Fig. 2a). Upon challenge with powdery mildew, both SNAP33 transcript and protein increased in abundance (Supplementary Figs 2a and 4a). SNAP33 assembled *in planta* in a pathogen-inducible SDS-resistant complex of similar size to the PEN1 complex and is capable of interacting *in vitro* with PEN1 (Supplementary Figs 2b and 3a). Analysis of transgenic *Arabidopsis* plants co-expressing functional fluorochrome-marked yellow fluorescent protein (YFP)–SNAP33 and cyan fluorescent protein (CFP)–PEN1 fusion proteins revealed co-localization and focal accumulation of both proteins at the plasma membrane beneath incipient powdery mildew entry sites (Supplementary Fig. 2c–f). Together, these findings suggest that SNAP33 cooperates with PEN1 at the plasma membrane. However, lethality of *snap33* mutants before flowering, associated with dwarfism, cytokinetic defects and necrotic leaf lesion formation, points to additional SNAP33 engagement(s) in essential cellular processes[10].

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Exocytosis is completed by vesicle fusion with target membranes and this is thought to be driven by complex formation of t-SNAREs with vesicle (v)-SNAREs, also called vesicle-associated membrane proteins (VAMPs)[4,11]. The *Arabidopsis* genome harbours 12 VAMP encoding genes, which fall into two subfamilies[8]. The detection of *in planta* PEN1-containing SNARE complex(es) prompted us to devise an *in vitro* systematic SNARE complex survey using purified *Arabidopsis* SNARE proteins that were expressed in *Escherichia coli*. We detected SDS-resistant SNARE complexes by incubating PEN1 and glutathione-*S*-transferase (GST)–SNAP33 bound to glutathione Sepharose 4B with various VAMPs. Sedimented bead-bound GST–SNAP33 was released and detected by immunoblot analysis using a GST antibody. GST–SNAP33 was able to form SDS-resistant and heat-sensitive complexes in the presence of PEN1 and each of four tested members of the VAMP72 subfamily, but not with VAMP711 (Fig. 1b). Barely detectable GST–SNAP33 complexes were seen in the presence of other VAMP71 subfamily members, VAMP712 and VAMP713 (not shown), suggesting that PEN1 associates preferentially with VAMP72 subfamily members. Importantly, when PEN1 was replaced by PEN1-3, complex formation was undetectable in the presence of VAMP722 and was reduced upon incubation with the other VAMP72 family members (VAMP721, VAMP724 and VAMP727; Fig. 1b). The presumed presence of all three SNARE proteins, PEN1, SNAP33 and VAMP722, in the SDS-resistant complex was validated by differential protein tagging (His_6–PEN1, GST–SNAP33 and HA–VAMP722) and immunoblot analysis using anti-His_5, anti-GST and anti-HA antibodies, respectively (Supplementary Fig. 3b, c). Pairwise association tests showed that PEN1 and VAMP722 do not associate alone under our experimental conditions and that PEN1-3 has a decreased affinity for SNAP33 (Supplementary Fig. 3a). Moreover, SDS-resistant complex formation required the presence of PEN1, SNAP33 and VAMP722 (Fig. 1c), demonstrating that all three SNARE proteins cooperate *in vitro*. This, and the lack of an *in planta* SDS-resistant PEN1-3 complex, identifies VAMP722 as potential major v-SNARE partner of PEN1. However, the residual PEN1-3 disease resistance activity to *B. graminis* (Supplementary Fig. 1a) suggests that either additional VAMP72 family members contribute to PEN1 function or that unstable PEN1-3–SNAP33–VAMP722 complexes retain *in planta* residual activity.

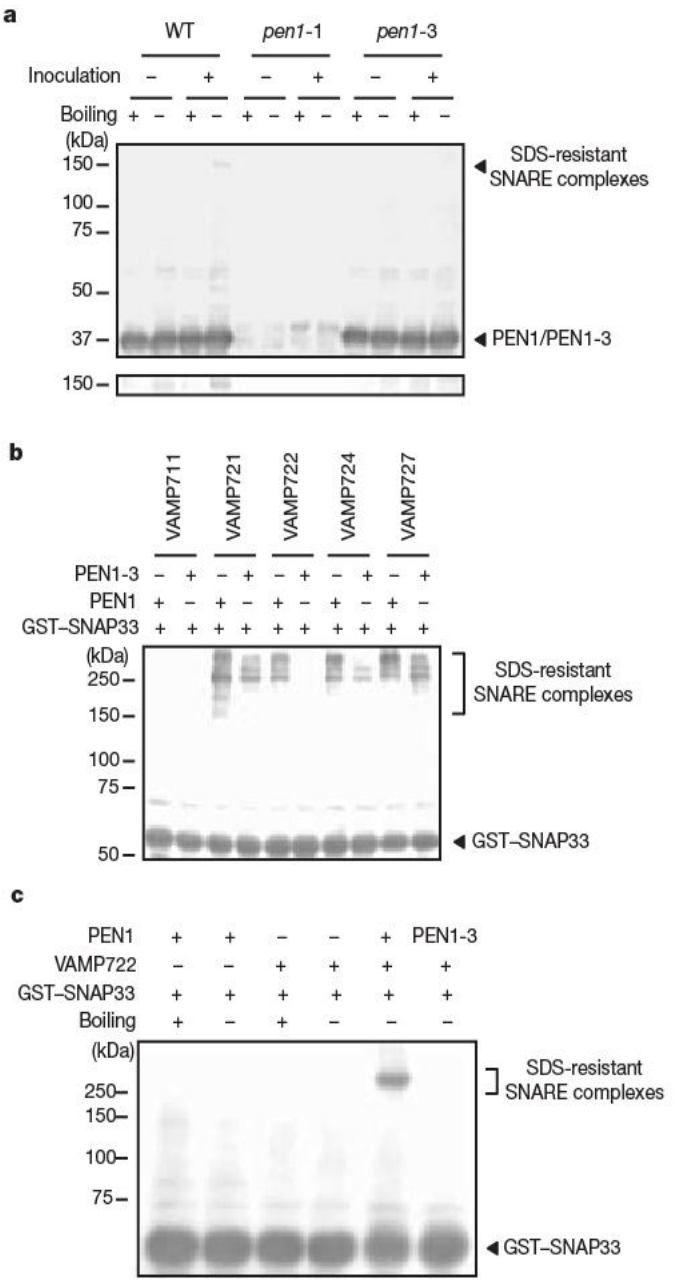

Figure 1 | PEN1-3 fails to form SDS-resistant SNARE complexes with VAMP722. **a**, A pathogen-inducible and SDS-resistant PEN1-containing complex is undetectable in *pen1*-3 plants. Proteins were extracted from leaves of wild-type (WT), *pen1*-1 or *pen1*-3 plants. Samples were taken from healthy leaves or those challenged with *B. graminis* 24 h after spore inoculation. Proteins (20 μg) were loaded on a 7.5% polyacrylamide gel and subjected to immunoblot analysis with anti-PEN1 antiserum. The lower panel shows a section of the same immunoblot corresponding to the PEN1-containing complex after over-exposure. **b**, Comparison of the SNARE complex-forming activity between PEN1 and PEN1-3. PEN1 or PEN1-3 (2 μM) were incubated with equimolar GST–SNAP33 and the indicated VAMPs. Bound proteins were pulled down with glutathione-Sepharose 4B and eluted with sample buffer containing SDS. Eluted proteins were loaded on a 7.5% polyacrylamide gel and subjected to immunoblot analysis with anti-GST antibody. Note that the *in vitro* SNARE complexes migrate at a different size to the *in vivo* PEN1-containing complex because of the presence of epitope tag. **c**, All three SNARE proteins are required for the formation of the SDS-resistant complex. The indicated proteins (2 μM) were incubated and SNARE complex formation was analysed as in **b**.

To address this, we used a genetic approach and selected homozygous T-DNA insertion lines for *VAMP712*, *VAMP714*, *VAMP721*, *VAMP722*, *VAMP723* and *VAMP727*. All T-DNA lines showed wild-type-like entry rates of *B. graminis* into leaf epidermal cells (not shown), indicating potential genetic redundancy. Because VAMP721 is most closely sequence-related to VAMP722, we aimed to isolate *vamp721 vamp722* double mutant lines. We generated plants that were homozygous mutant for either *VAMP721* or *VAMP722* and heterozygous for the respective other gene and analysed the genotypes of their progeny from selfings (Supplementary Table 1). Among 667 progeny tested, however, there were no plants that were homozygous mutant for both genes. This suggests fully penetrant lethality of *vamp721 vamp722* double mutants and essential as well as redundant wild-type gene functions in development. Moreover, closer inspection of the segregation ratios of progeny genotypes recovered from the two parental lines $VAMP721^{-/-}$ $VAMP722^{+/-}$ and $VAMP721^{+/-}$ $VAMP722^{-/-}$ indicated a deviation from the expected 1:2 segregation, but this was statistically significant only for the latter ($P = 0.027$; Supplementary Table 1). This reveals an additional gametophytic activity that is normally not apparent because of the redundant *VAMP721* and *VAMP722* function in diploid plants. Interestingly, *VAMP721* and *VAMP722* appear to act in compensating pathways as VAMP722 transcript abundance was markedly increased in the homozygous *vamp721* single mutant background. A similar though less dramatic compensatory VAMP721 transcript increase was found in homozygous *vamp722* plants (Supplementary Figs 5c and 7a). Transgenic $35S::RNAi_{VAMP722}$ gene silencing lines in which both *VAMP721* and *VAMP722* transcript levels were specifically and severely reduced grew normally for about two weeks. Thereafter, necrotic leaf lesions appeared and the plants exhibited an overall dwarf stature that was accompanied by a reduction of leaf mesophyll cell size of approximately 60% (Supplementary Fig. 4b–d). Because the same phenotype

appears with the same timing in *pen1 syp122* double mutants[6,12], this might indicate overlapping VAMP721/VAMP722 and PEN1/SYP122 functions.

Next we generated transgenic *Arabidopsis* lines for conditional *VAMP721 VAMP722* co-silencing using the ethanol-inducible *alc* system[13]. Using low concentrations of ethanol vapour applied to two-week-old plants and subsequent challenge with *B. graminis* spores, we identified multiple lines in which fungal entry rates were significantly elevated in an inducer-dependent manner; that is, showing a *pen1* phenocopy (Fig. 2a and Supplementary Fig. 5a–c). Because elevated fungal entry was observed without recognizable pleiotropic plant growth effects and correlated with a reduction of *VAMP721* and *VAMP722* transcript levels (Supplementary Fig. 5c), this provides genetic evidence for a *VAMP721/VAMP722* function in disease resistance that can be uncoupled from their essential role in development.

For direct *in planta* VAMP721/722 immunodetection we generated an anti-VAMP721/722 antibody using the cytosolic part of VAMP722 (VAMP722ΔTM). This antibody specifically detected both VAMP721 and VAMP722 sharing 96% identical amino-acid residues (Fig. 2b). Analysis of the steady-state level of both proteins in leaf extracts showed dependence on gene dosage and revealed that the relative amount of VAMP722 apparently greatly exceeds that of VAMP721 (Fig. 2b). We used protein extracts from transgenic plants expressing functional SNAP33–myc driven by a 2.6 kilobase (kb) native promoter sequence in a *snap33* background[10] for co-immunoprecipitation experiments with the anti-myc antibody. Anti-myc immune complexes contained both PEN1 and VAMP721/VAMP722 (Fig. 2c). Likewise, immunoprecipitation experiments with extracts from transgenic plants expressing functional green fluorescent protein (GFP)–PEN1 driven by the 35S promoter in a *pen1*-1 background[1] revealed in anti-GFP immune complexes the presence of SNAP33 and VAMP721/VAMP722 (Fig. 2c). Together this provides direct evidence for *in planta* PEN1–SNAP33–VAMP721/VAMP722 ternary SNARE complexes.

To investigate the subcellular localization of VAMP722 in living plant cells in response to fungal attack, we generated transgenic plants expressing GFP fused to VAMP722 (GFP–VAMP722) under the control of 1.2 kb native 5′ regulatory *VAMP722* sequences in the *vamp722* background. These lines showed a pathogen-inducible accumulation of functional GFP–VAMP722, as indicated by immunoblot analysis of leaf protein extracts and restored resistance to fungal entry (Supplementary Figs 4a and 7b). Confocal microscopy revealed focal accumulation of GFP-fluorescent intracellular compartments in single attacked leaf epidermal cells directly beneath *B. graminis* appressoria (Fig. 3b). Whereas the polarized accumulation of GFP–VAMP722 is similar to the focal concentration of plasma-membrane-resident CFP–PEN1[12,14] and YFP–SNAP33 at incipient entry sites (Supplementary Fig. 2c–f), the former marks a different compartment(s). GFP–VAMP722-tagged structures differing in size were distinguishable (Fig. 3b). This is reminiscent of compound exocytosis in mammals in which vesicles fuse with each other

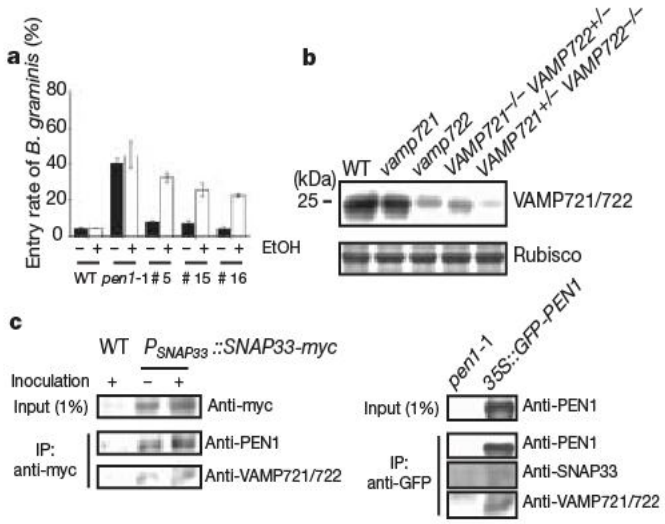

Figure 2 | VAMP721 and VAMP722 are required for PEN1-mediated immune responses. **a**, Compromised pre-invasion resistance after conditional silencing of *VAMP721* and *VAMP722* expression. The entry rate of *B. graminis* is increased in the *alc::RNAi*$_{VAMP722}$ silencing plants in an ethanol-dependent manner. Leaves of the indicated plants were harvested at 48 h post-inoculation with *B. graminis* spores and analysed by aniline blue staining and ultraviolet-light microscopy. Error bars indicate standard errors ($n = 4$). **b**, Gene-dosage-dependent control of VAMP721/722 steady-state levels. VAMP721/722 protein levels were analysed by leaf proteins extracts from the indicated plant genotypes by immunoblot using anti-VAMP721/722 antibody. Note that the VAMP721/722 level in *VAMP721*$^{-/-}$ *VAMP722*$^{+/-}$ or *VAMP721*$^{+/-}$ *VAMP722*$^{-/-}$ plants is lower than in *vamp721* or *vamp722* plants, respectively. **c**, *In planta* interactions between PEN1, SNAP33 and VAMP721/722. Immunoprecipitation (IP) was performed with an anti-myc (left) or anti-GFP (right) antibody using protein extracts from the indicated plant genotypes. Leaf material was harvested 24 h after *B. graminis* spore inoculation or from healthy plants. Immune complexes were analysed by immunoblot using anti-PEN1, anti-SNAP33 or anti-VAMP721/722 antibody. Protein extracts from wild-type or *pen1*-1 plants were used as negative control.

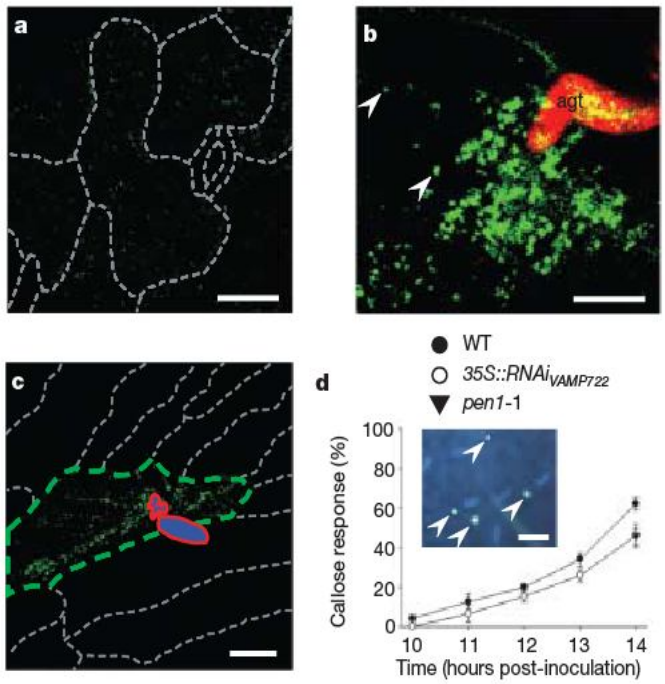

Figure 3 | Focal accumulation and directed movement of VAMP722-containing intracellular compartments to powdery-mildew entry sites. **a**, Random distribution of GFP-tagged compartments in a cluster of epidermal pavement and stomata cells (grey dashed lines mark cell margins) of a healthy plant. The image combines stack signals of ten confocal sections at 1.91 μm intervals. Scale bar, 25 μm. **b**, Focal accumulation of mobile punctuate structures labelled by GFP–VAMP722 (green) to a fungal entry site of a leaf epidermal cell. Note different sizes of the punctuate compartments (arrowheads). The picture was taken 15 h after inoculation with *B. graminis* spores (red). agt, appressorial germ tube. Scale bar, 10 μm. **c**, Cell-autonomous expression of GFP–VAMP722 and directed movement of GFP-fluorescent compartments to a fungal entry site on an epidermal midrib cell. The picture was taken 13 h after inoculation with *E. pisi* spores (red-lined blue). Note that the signal of GFP–VAMP722 is only detectable in the attacked cell (green dashed lines; grey dashed lines mark neighbouring epidermal cells). Scale bar, 40 μm. **d**, Retarded callose deposition in *35S::RNAi*$_{vamp722}$ silencing plants. Callose deposition was visualized by aniline blue at the appressorial germ tubes (arrowheads in inset) and was determined at the indicated time points after *B. graminis* spore inoculation. Filled circle, wild type; open circle, *35S::RNAi*$_{vamp722}$ silencing plants; filled triangle, *pen1*-1. Error bars indicate standard errors ($n = 3$). Scale bar, 50 μm.

before their fusion with the plasma membrane[15] and/or represents aggregation of the punctuate compartments seen in healthy plants (Fig. 3a). In response to challenge with *E. pisi*, GFP–VAMP722 fluorescence at the leaf mid-vein region was detectable only in single attacked epidermal cells and characterized by a directed movement of GFP–VAMP722-tagged compartments to the fungal invasion site (Fig. 3c). In contrast, epidermal pavement cells of healthy unchallenged plants showed low abundance and random distribution of fluorescent structures (Fig. 3a). Collectively, these data are consistent with a pathogen-inducible VAMP722-mediated delivery of secretory compartments to incipient fungal entry sites. Pathogen-induced *de novo* cell-wall biosynthesis beneath fungal appressoria is delayed in *pen1*-1 plants compared with wild type[12]. We investigated in time-course experiments PMR4/GSL5-mediated callose formation[16,17] as a marker of this process in wild type, *pen1*-1 and *35S::RNAi*$_{VAMP722}$ transgenic lines in which *VAMP721* and *VAMP722* are constitutively co-silenced. The incidence of callose formation in the silencing lines was lower relative to wild type at all inspected time points and similar to *pen1*-1 plants (Fig. 3d). This hints to a common role for VAMP721/VAMP722 and PEN1 in focal secretion at fungal entry sites and is consistent with the hypothesis that PEN1 syntaxin acts through an authentic SNARE complex by association with VAMP721/VAMP722.

Barley HvROR2 and HvSNAP34 are orthologues of *Arabidopsis* PEN1 and SNAP33, and are known to restrict entry of *B. graminis* into epidermal cells of barley leaves[1]. We searched the HarvEST (http://harvest.ucr.edu/) barley EST database (assembly 31) and identified one unigene contig, Hv4993, that encodes a presumptive orthologue (termed HvVAMP721) of *Arabidopsis* VAMP721/VAMP722 (Supplementary Fig. 6a). As in *Arabidopsis*, each of fluorescently labelled HvVAMP721, HvROR2 and HvSNAP34 accumulate at incipient powdery-mildew entry sites (not shown). Transient single-cell co-expression of fluorophore-tagged fusion proteins coupled with Förster resonance energy transfer analysis based on fluorescence lifetime imaging (FRET–FLIM) revealed pairwise *in vivo* interactions between HvROR2, HvSNAP34 and HvVAMP721 (Supplementary Fig. 6b), thereby supporting their capacity to cooperate *in planta*. Concentration of these barley SNARE proteins at fungal entry sites and *in vivo* interactions in the absence of the pathogen suggest that their dual function in immunity and cellular housekeeping is evolutionarily conserved.

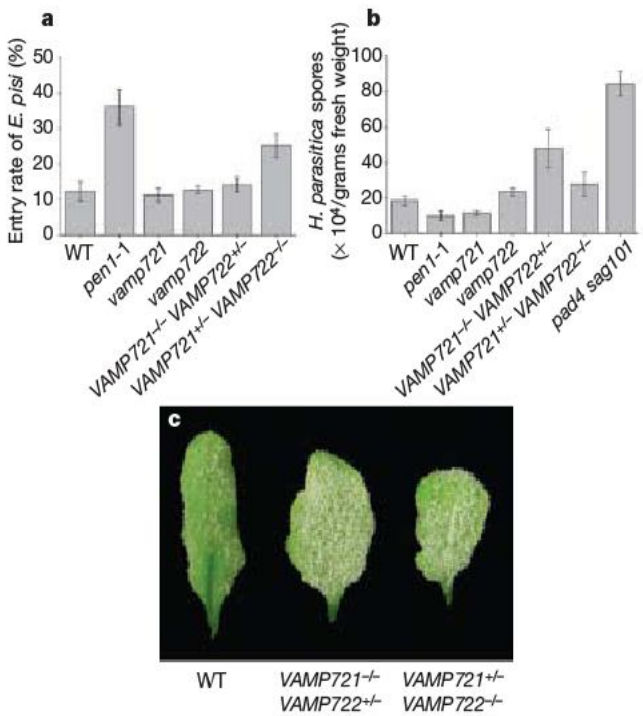

Figure 4 | Compromised pre- and post-invasion resistance by lowered gene dosage of *VAMP721* and *VAMP722*. **a**, Increased entry rate of *E. pisi* in *VAMP721*$^{+/-}$ *VAMP722*$^{-/-}$ plants. The entry rate was microscopically scored at seven days post-inoculation with *E. pisi* spores. Error bars indicate standard errors ($n = 3$). **b**, Hyper-susceptibility of *VAMP721*$^{-/-}$ *VAMP722*$^{+/-}$ plants to *H. parasitica*. The susceptibility was analysed by counting conidiospore numbers at six days post-inoculation with *H. parasitica* spores. Error bars indicate standard errors ($n = 3$). **c**, Hyper-susceptibility of both *VAMP721*$^{+/-}$ *VAMP722*$^{-/-}$ and *VAMP721*$^{-/-}$ *VAMP722*$^{+/-}$ plants to *G. orontii*. The susceptibility to *G. orontii* was macroscopically analysed with the indicated genotypes at ten days post-inoculation. Note that inoculated leaves of *VAMP721*$^{+/-}$ *VAMP722*$^{-/-}$ and *VAMP721*$^{-/-}$ *VAMP722*$^{+/-}$ plants are more densely covered with *G. orontii* mycelium and sporangiophores compared with wild type.

We noted that the compensatory expression of *VAMP722* or *VAMP721* is abolished in *VAMP721*$^{-/-}$ *VAMP722*$^{+/-}$ and *VAMP721*$^{+/-}$ *VAMP722*$^{-/-}$ *Arabidopsis* plants (Supplementary Fig. 7a). Moreover, the transcript levels in these genotypes upon challenge with *B. graminis* are even lower than those in wild type and *vamp721* or *vamp722* single mutants (Supplementary Fig. 7a). This and the dependence on gene dosage of VAMP721/722 steady-state levels (Fig. 2b) prompted us to examine potentially impaired non-host disease resistance to *B. graminis* in *VAMP721*$^{+/-}$ *VAMP722*$^{-/-}$ and *VAMP721*$^{-/-}$ *VAMP722*$^{+/-}$ genotypes. Only the former genotype allowed enhanced fungal entry into leaf epidermal cells, which was similar to the phenotype seen upon conditional co-silencing of *VAMP721* and *VAMP722* (Supplementary Fig. 5). Likewise, inoculation of the same genotypes with non-adapted *E. pisi* spores allowed elevated fungal entry rates only on *VAMP721*$^{+/-}$ *VAMP722*$^{-/-}$ plants (Fig. 4a). Thus, in the absence of the entry control function of *VAMP722*, *VAMP721* becomes haplo-insufficient to both tested non-adapted powdery mildews. Intriguingly, *VAMP721*$^{-/-}$ *VAMP722*$^{+/-}$ plants were hyper-susceptible to the virulent oomycete *Hyaloperonospora parasitica* (isolate Noco2), as indicated by elevated spore production at six days post-inoculation (Fig. 4b). This reveals *VAMP722* haploinsufficiency in a homozygous *vamp721* background and demonstrates that both VAMPs contribute to disease resistance against different pathogen classes. To test whether a similar resistance function restricts the growth of an adapted powdery mildew, we inoculated the respective genotypes with spores of *Golovinomyces orontii*. Both *VAMP721*$^{+/-}$ *VAMP722*$^{-/-}$ and *VAMP721*$^{-/-}$ *VAMP722*$^{+/-}$ plants were hyper-susceptible to *G. orontii* (Fig. 4c and Supplementary Fig. 8a). This demonstrates that immune responses to non-adapted and adapted pathogens engage common secretory components and that VAMP721 and VAMP722 contribute to a wider range of defence responses than PEN1. However, bacterial growth of *Pseudomonas syringae* DC3000 was indistinguishable between the tested plant genotypes (Supplementary Fig. 8b). This and the disease resistance function of *Nicotiana benthamiana Nb*SYP132 against *P. syringae*[18], a syntaxin that belongs to a different SYP subfamily than PEN1 and SYP122, suggest that plants use a separate secretory pathway to restrict bacterial growth.

To our knowledge, we provide for the first time biochemical and genetic evidence for ternary SNARE complex functions in plants. Our findings show that PEN1–SNAP33–VAMP721/VAMP722 complexes are required for immune responses to powdery mildew/oomycete pathogens, and that VAMP721 and VAMP722 act redundantly during development, thereby revealing dual functions in separate biological processes. Reduced gametophytic transmission of the *vamp721* and/or *vamp722* mutation, the lethality of *vamp721 vamp722* double mutants, and plant dwarfism in *35S::RNAi*$_{VAMP722}$ gene silencing lines (Supplementary Table 1 and Supplementary Fig. 4b–d) probably reflect perturbation of an ancient default secretory pathway, which is essential for viability of plant cells. Both *35S::RNAi*$_{VAMP722}$ gene silencing lines and *pen1 syp122* double mutants exhibit severe dwarfism[6,12] (Supplementary Fig. 4c). However, unlike the lethal *vamp721 vamp722* genotype, *pen1 syp122* double mutants can be propagated as true breeding lines[6,12]. Thus, the

default activity of VAMP721/VAMP722 cannot act solely through PEN1 and SYP122, but is likely to engage additional syntaxins. Similarly, the immune response function of VAMP721/VAMP722 cannot act solely through PEN1 because the VAMPs contribute to a wider range of defence responses than PEN1 (Fig. 4). Collectively, our findings suggest that *Arabidopsis* has co-opted the pre-existing VAMP721/VAMP722-dependent secretion apparatus for immune responses to pathogens. In one scenario, the default transport pathway could be re-used to deliver pathogen-inducible cargo. The broad-spectrum activity of the identified VAMPs against an ascomycete and oomycete parasite (Fig. 4) and the delay in pathogen-inducible cell wall reinforcement in *35S::RNAi*$_{VAMP722}$ transgenic lines (Fig. 3d) predict broadly effective antimicrobials[19] and cell-wall components as candidate cargo.

Focal concentration of PEN1, SNAP33 and VAMP722 (Fig. 3b, c and Supplementary Fig. 2c–f) at pathogen entry sites and the resistance function of the corresponding SNARE complex provide a missing link to synthesize observations on seemingly diverse pathogen-induced cellular re-distribution events. This involves the disassembly and polar re-assembly of the actin cytoskeleton[20], polar re-distribution of intracellular organelles[21,22] including PEN2-containing peroxisomes[2], and the formation of lipid raft-like plasma membrane microdomains underneath pathogen entry sites[14]. This, and the requirement of the secretory SNARE complex for pre-invasion resistance, are mechanistically strikingly similar to the formation of the immunological synapse in vertebrates for effective immune responses through focal secretion[23,24]. Cytotoxic T lymphocytes kill antigen-presenting cells by secreting perforin (also called cytolysin) and serine proteases upon recognition of non-self peptides presented by plasma-membrane-resident major histocompatibility complexes[25]. In interactions with the non-adapted *B. graminis* and *E. pisi* powdery mildews, the resistance response restricts fungal entry into leaf epidermal cells. Because of the focal accumulation of PEN1, SNAP33 and VAMP722 beneath appressoria, we conclude that focal secretion terminates pathogenesis in a process that resembles the immunological synapse formation. Unlike powdery mildews that exclusively infect epidermal cells[22], *H. parasitica* retrieves nutrients mainly from mesophyll cells by differentiation of ramified hyphae in the apoplastic space of mesophyll tissue[21]. Thus, the observed hyper-susceptibility to *G. orontii* and *H. parasitica* (Fig. 4b, c) implies impaired VAMP721/722 secretory activity both in epidermal and mesophyll cells. Activated T cells release different cargos by distinct secretory pathways: besides focal secretion through the immunological synapse, multi-directional secretion is used to promote inflammation[26]. Because adapted powdery mildews such as *G. orontii* or *E. cichoracearum* have overcome pre-invasion resistance[2,6], hyper-susceptibility to *G. orontii* could be partly due to reduced non-directional VAMP721/722 secretory activity from neighbouring cells.

METHODS SUMMARY

Plant material. All plants used for experiments were *Arabidopsis* Col-0, except *pen1*-3 whose background was *Arabidopsis* Col-3 *gl1*. All plants used were soil grown in growth chambers at 20–23 °C with a 10 h photoperiod and a light intensity of about 150 $\mu E\,m^{-2}\,s^{-1}$. For gene silencing experiments, two-week-old plants were used. The induction was performed in a closed chamber (50 cm × 30 cm × 20 cm) by ethanol vapour for 12 days. Gene silencing was periodically induced with alternating two-day intervals with and without ethanol. Total protein extracts were prepared from plant leaves by grinding the tissue in liquid nitrogen and suspending extracts in 1 × PBS (pH 8.0) containing 1% Triton X-100. The solution was centrifuged and the supernatant used for further experiments.

To isolate homozygous *vamp712* (At2g25340), *vamp714* (At5g22360), *vamp721* (At1g04750), *vamp722* (At2g33120), *vamp723* (At2g33110) and *vamp727* (At3g54300) mutants, GABI_054H03, GABI_844B05, SALK_037273, SALK_119149, GABI_839H05 and GABI_060G05 lines were obtained from ABRC and GABI-Kat and analysed by PCR and DNA sequencing. To generate GFP–VAMP722 transgenic lines, GFP was fused in-frame to the amino (N) terminus of full-length *VAMP722* and downstream of a 1.2 kb fragment of VAMP722 5′ regulatory sequence ($P_{VAMP722}$::GFP–VAMP722). To generate transgenic plants expressing CFP–PEN1 and YFP–SNAP33, CFP or YFP was fused in-frame to the N terminus of full-length PEN1 or SNAP33, respectively, downstream of the 35S promoter (35S::CFP–PEN1 or 35S::YFP–SNAP33). Transgenic plants co-expressing both CFP–PEN1 and YFP–SNAP33 were obtained by crossing the above-mentioned transgenic lines. To generate *VAMP721/VAMP722*-silencing plants, complementary DNA (cDNA) corresponding to the cytoplasmic region of VAMP722 was inserted downstream of either the 35S or *alc* promoter as inverted repeats. All transgenic plants were generated by transformation mediated by *Agrobacterium tumefaciens*.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions C.K., R.P., V.L. and P.S.-L. designed all experiments. C.K., C.N., S.P., H.S.Y., U.L., M.H. S.B., M.S., M.K., H.R. and F.E.K. performed the experiments. C.K., V.L., G.J., R.P. and P.S.-L. analysed the data. C.K. and P.S.-L. wrote the paper.

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LETTERS

Two levels of protection for the B cell genome during somatic hypermutation

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Somatic hypermutation introduces point mutations into immunoglobulin genes in germinal centre B cells during an immune response. The reaction is initiated by cytosine deamination by the activation-induced deaminase (AID) and completed by error-prone processing of the resulting uracils by mismatch and base excision repair factors[1]. Somatic hypermutation represents a threat to genome integrity[2] and it is not known how the B cell genome is protected from the mutagenic effects of somatic hypermutation nor how often these protective mechanisms fail. Here we show, by extensive sequencing of murine B cell genes, that the genome is protected by two distinct mechanisms: selective targeting of AID and gene-specific, high-fidelity repair of AID-generated uracils. Numerous genes linked to B cell tumorigenesis, including *Myc*, *Pim1*, *Pax5*, *Ocab* (also called *Pou2af1*), *H2afx*, *Rhoh* and *Ebf1*, are deaminated by AID but escape acquisition of most mutations through the combined action of mismatch and base excision repair. However, approximately 25% of expressed genes analysed were not fully protected by either mechanism and accumulated mutations in germinal centre B cells. Our results demonstrate that AID acts broadly on the genome, with the ultimate distribution of mutations determined by a balance between high-fidelity and error-prone DNA repair.

Previous studies identified four non-immunoglobulin genes (*BCL6*, *FAS*, *CD79A*, *CD79B*) as somatic hypermutation targets in normal human B cells[3–6], as well as several genes (including *MYC*, *PIM1*, *PAX5* and *RHOH*) that are mutated in certain human B cell tumours[7]. Combined with studies of reporter genes in transformed B cell lines indicating that AID can trigger mutations at many locations in the genome[8,9], this raised the possibility that the genome accumulates a substantial mutation load as a result of somatic hypermutation. To investigate this, we performed a large-scale DNA sequencing analysis of non-immunoglobulin genes from Peyer's patch B cells of ~6-month-old wild-type and AID-deficient littermate mice. Because of the established link between transcription and somatic hypermutation[10], we focused initially on genes that are expressed in germinal centre B cells. Other criteria for gene selection are described in the Methods, and complete mutation data are provided in Supplementary Table 1. A ~1-kilobase (kb) segment of each gene, located in the 1.5-kb region downstream of the major transcription start site, was amplified, sequenced and analysed for point mutations, with 70,000–100,000 nucleotides of sequence information collected for each gene in most cases.

The mutation frequency for 118 expressed genes analysed from wild-type B cells ranged from zero to nearly 90×10^{-5} mutations per base pair (bp) (Fig. 1). Analysis of 31 genes from $Aid^{-/-}$ B cells (Fig. 1, white bars), which undergo extensive proliferation but lack somatic hypermutation[11], yielded an average background mutation frequency attributable to the sequencing procedure of 1.6×10^{-5} mutations per bp. For each of these 31 genes, the mutation frequency was higher in wild-type than in $Aid^{-/-}$ B cells (Fig. 1 and Supplementary Fig. 2). Although the mutation frequencies in wild-type mice were well below that measured at the immunoglobulin heavy chain (*Igh*) JH4 intronic region (2.2×10^{-2} mutations per bp), we found that many genes were significantly mutated compared to the $Aid^{-/-}$ background value. Genes were divided into three groups: group I, consisting of 23 (19%) of the most highly mutated genes ($q < 0.05$; see Methods); group II, consisting of 25 (21%) genes with intermediate mutation frequencies ($0.05 \leq q < 0.2$); and group III, consisting of 70 (60%) infrequently mutated genes ($q \geq 0.2$). Mutations in group I genes were highly biased towards the somatic hypermutation hotspot sequence RGYW/WRCY and exhibited a significant A greater than T mutation bias on the non-transcribed strand (Table 1). Both of these biases could also be detected in group II genes, although hotspot targeting was not statistically significant (Table 1). The frequency of mutations in group III genes was not statistically different from the background. We conclude that one-quarter to one-third of the expressed genes analysed, including almost all group I and some group II genes, accumulate mutations as a result of somatic hypermutation. This stands in contrast to results obtained from 17 genes predicted not to be expressed in germinal centre B cells, none of which exhibited a significant level of mutation ($q > 0.2$) (Fig. 1, inset).

Consistent with previous studies[3,4], *Bcl6* was the most highly mutated non-immunoglobulin gene examined. Novel somatic hypermutation targets were identified, including *Cd83*, *Btg1*, the tyrosine kinases *Blk* and *Syk*, the B cell transcription factor *Ocab*, and *Ung* (Fig. 1). In addition, the oncogenes *Pim1*, *Rhoh*, *Pax5* and *Myc*, previously reported not to undergo somatic hypermutation in normal human B cells[7,12], were found to accumulate detectable levels of mutations. In contrast, *Cd79a* and the tumour suppressor genes *Trp53* (which encodes p53), *H2afx* and *Fas* were not detectably mutated. Mutated genes are scattered widely throughout the genome (Supplementary Fig. 3), indicating that genomic location does not readily explain somatic hypermutation targeting.

Although numerous transcribed genes are targets of somatic hypermutation, our data suggest that much of the genome is protected from substantial mutation accumulation. This could be accomplished solely through selective targeting of AID, in which case the genomic distribution of mutations would directly reflect the sites of AID action. However, it is also possible that AID acts more widely

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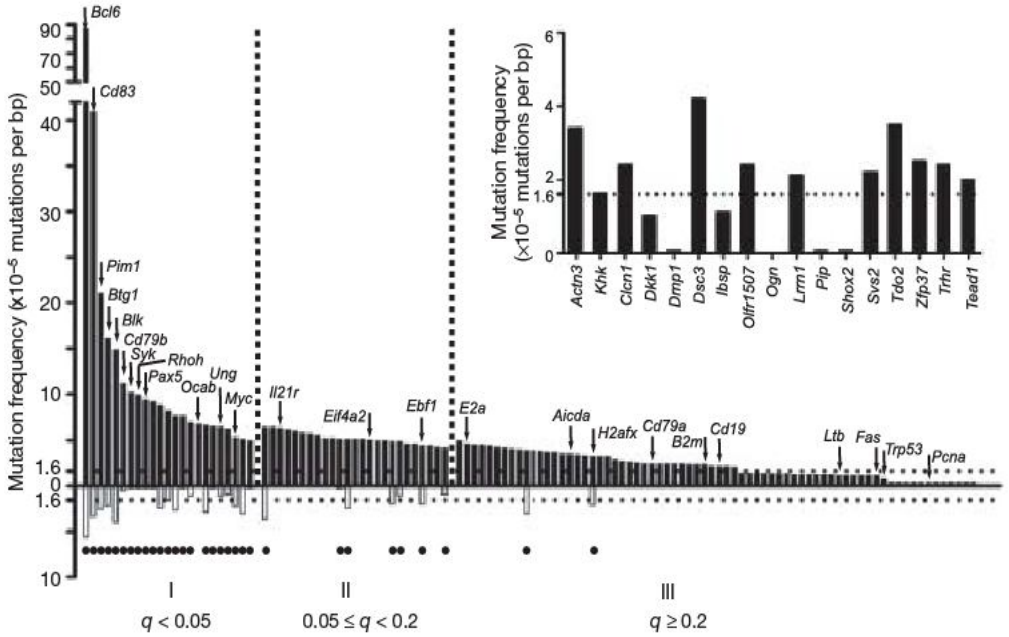

Figure 1 | Gene mutation frequencies in wild-type and *Aid*$^{-/-}$ B cells. Results for wild-type (black bars) and *Aid*$^{-/-}$ (white bars) B cells are plotted above and below the *x*-axis, respectively. Black dots indicate the 31 genes sequenced from *Aid*$^{-/-}$ B cells. Inset, results for genes predicted not to be expressed in germinal centre B cells. Horizontal dotted lines indicate the background mutation frequency. *q* values reflect correction for multiple testing. Because *q* values are influenced by the number of nucleotides sequenced, which varies from gene to gene, a group II (or III) gene with fewer sequenced nucleotides can have a higher mutation frequency than a group I (or II) gene. DNA preparations were obtained from cells pooled from 3–5 mice (wild type, >10 pools; *Aid*$^{-/-}$, 6 pools), and the number of independent DNA preparations analysed for each gene is indicated in Supplementary Table 1.

than suggested by the mutation data, with portions of the genome protected from somatic hypermutation by high-fidelity repair of AID-generated uracils. To test this possibility, we sequenced non-immunoglobulin genes from Peyer's patch B cells of 4-month-old *Msh2*$^{-/-}$*Ung*$^{-/-}$ double knockout mice, which are deficient in important components of the mismatch repair and uracil base excision repair pathways[13]. Msh2/Msh6 and Ung are involved in both the creation of mutations during somatic hypermutation and high-fidelity repair of U•G mismatches[1] (Fig. 2a, inset), and in their absence, DNA replication over unrepaired uracils reveals the 'footprint' of AID action as a distinctive pattern of C•G to T•A transition mutations[13–15].

Of the 83 expressed genes sequenced from double knockout Peyer's patch B cells (Fig. 2a), 36 were chosen from among those sequenced from wild-type B cells (Fig. 2b) and 47 were new genes involved in tumorigenesis or B cell signalling. Complete mutation data are provided in Supplementary Table 2. Thirty-six genes (43%) were highly significantly mutated above the experimental background and were divided into group A ($q < 10^{-6}$) or group B ($10^{-6} \leq q < 0.01$), with the remaining genes referred to as group C (Fig. 2a). Several criteria were used to distinguish between AID-dependent and AID-independent mutations. Group A genes exhibited a strong bias for C•G to T•A transition mutations and enrichment for mutations in AID hotspots (Fig. 2a and Table 1). Notably, mutations in group B and group C genes were also strongly enriched in AID hotspots (Fig. 2a and Table 1). This includes *Trp53*, for which 5 out of 8 mutations detected were in hotspots. Most importantly, sequencing of seven group A genes from *Aid*$^{-/-}$*Msh2*$^{-/-}$*Ung*$^{-/-}$ triple knockout germinal centre B cells and of *Myc* and *H2afx* from naive double knockout B cells (which do not express AID) revealed mutation frequencies indistinguishable from background (Fig. 2b, inset; see also Supplementary Table 2). We conclude that virtually all group A mutations, most group B mutations, and a substantial proportion of group C mutations are the

Table 1 | Summary of mutation data from wild type and mutant B cells

Genotype	Genes	Number of mutations (unique mutations)*	Mutations at C/G (%)†	Transitions at C/G (%)‡	A:T ratio§	Hotspot fold over random‖
Wild type	*JH4*	675 (459)	44	59	1.8 ($P < 10^{-7}$)	3.2 ($P < 10^{-33}$)
	Bcl6	204 (193)	47	65	1.2 ($P = 0.45$)	3.1 ($P < 10^{-8}$)
	Cd83	113 (92)	66	74	2.6 ($P < 0.01$)	4.4 ($P < 10^{-16}$)
	Other group I ($q < 0.05$)	324 (313)	65	84	1.8 ($P < 0.01$)	2.5 ($P < 10^{-13}$)
	Group II ($0.05 \leq q < 0.2$)	105 (104)	65	84	2.3 ($P < 0.02$)	1.4 ($P = 0.14$)
	Group III ($q \geq 0.2$)	112 (111)	67	78	1.0 ($P = 0.54$)	1.0 ($P = 0.52$)
Aid$^{-/-}$	31 genes	46 (45)	58	79	1.0 ($P = 0.61$)	0.9 ($P = 0.68$)
Msh2$^{-/-}$*Ung*$^{-/-}$ double knockout	*JH4*	852 (205)	99	100	ND	3.9 ($P < 10^{-100}$)
	Bcl6	144 (89)	100	100	ND	3.0 ($P < 10^{-10}$)
	Myc	363 (218)	98	99	ND	4.8 ($P < 10^{-92}$)
	Other group A ($q < 10^{-6}$)	834 (627)	97	98	ND	4.2 ($P < 10^{-100}$)
	Group B ($10^{-6} \leq q < 10^{-2}$)	177 (163)	84	86	ND	2.9 ($P < 10^{-12}$)
	Group C ($q \geq 10^{-2}$)	117 (116)	71	87	ND	2.8 ($P < 10^{-6}$)

* Total number of mutations (number of unique mutations).
† Percentage of mutations at C or G (corrected for base composition).
‡ Percentage of C or G mutations that are transitions.
§ Number of mutations at A divided by the number of mutations at T on the non-template strand (corrected for base composition). ND, not determined.
‖ Fold enrichment of mutations in AID hotspots RGYW/WRCY.

direct result of AID deamination, and hence that AID acts extremely widely on transcribed genes in the mouse genome.

We (Supplementary Fig. 5) and others[13,14] have observed that mutations accumulate to roughly equal levels (within twofold) in the *Igh* JH4 intronic region from wild-type and Msh2/Ung or Msh6/Ung double-deficient mice, suggesting that at immunoglobulin loci, error-prone repair frequently converts AID-generated uracils into mutations. Two group I genes (*Bcl6*, *Cd83*) follow this pattern (Fig. 2b), with similar mutation frequencies in double knockout and wild-type mice (double knockout:wild type ratio <2). Strikingly, however, numerous genes were identified in which the double knockout:wild type mutation ratio was substantially higher, including *Myc* and *H2afx* (double knockout:wild type ratio ~17), *Pax5* (~8.5), *Ebf1* (~8) and *Ocab* (~7) (Fig. 2b). At these loci it is evident that high-fidelity repair predominates, in some cases (for example, *H2afx*) to such an extent that all evidence of AID action is erased. The marked increase in mutation frequency of these genes in double knockout B cells is not due to increased expression of these genes in double knockout as compared to wild-type cells (Supplementary Fig. 6). We conclude that a wide variety of genes, scattered throughout the genome (Supplementary Fig. 3), are targeted by AID in normal germinal centre B cells but shielded from substantial mutation accumulation by high-fidelity repair (Supplementary Fig. 1).

To determine which repair pathway provides high-fidelity protection for the genome, we determined the mutation frequency of seven AID target genes in Peyer's patch B cells of $Ung^{-/-}$ and $Msh2^{-/-}$ single knockout mice. Good agreement was observed in the mutation levels between independent preparations of DNA (Supplementary Fig. 5). Mutation levels in $Msh2^{-/-}$ mice were similar to or well below those seen in wild-type mice (Fig. 3), demonstrating that base excision repair is capable of efficient high-fidelity repair of AID-generated uracils. In contrast, a majority of the genes analysed exhibited substantial mutation levels in $Ung^{-/-}$ mice (Fig. 3),

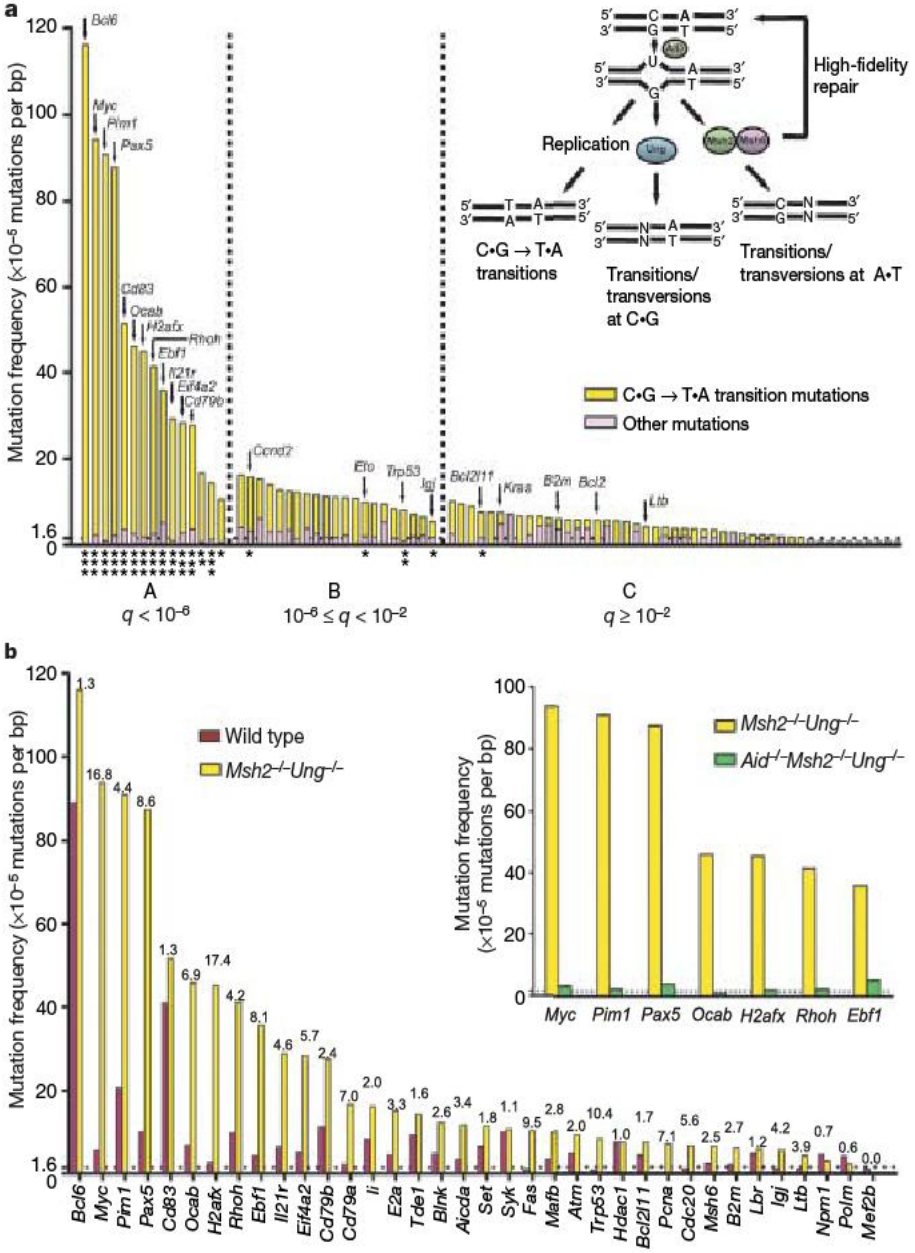

Figure 2 | Mutation frequencies in $Msh2^{-/-}Ung^{-/-}$ double knockout B cells. **a**, Double knockout B cells: yellow (C•G to T•A transition mutations), pink (other mutations). Statistical significance of hotspot enrichment: asterisk, $q<0.05$; double asterisk, $q<0.01$; triple asterisk, $q<0.001$. Inset, simplified model for somatic hypermutation[1]. **b**, Comparison of wild type (red bars) and double knockout (yellow bars) mutation frequencies. The number above each yellow bar is the double knockout:wild type mutation frequency ratio. Inset, comparison of mutation frequencies for seven genes from double knockout (yellow bars) and from $Aid^{-/-}Msh2^{-/-}Ung^{-/-}$ triple knockout (green bars) B cells. DNA preparations were obtained from single mice (double knockout, 6 mice; triple knockout, 2 mice), and the number of independent DNA preparations analysed for each gene is indicated in Supplementary Table 2.

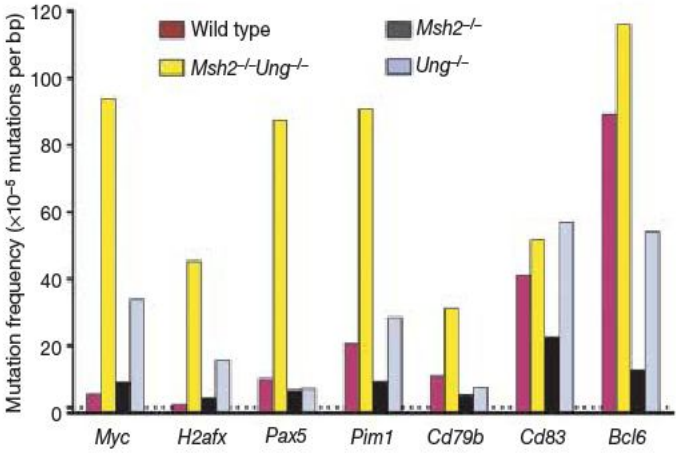

Figure 3 | Mutation frequencies in Msh2-deficient or Ung-deficient B cells. Mutation frequencies of selected genes from wild type (red bars), double knockout (yellow bars), $Msh2^{-/-}$ (black bars) and $Ung^{-/-}$ (blue bars) mice are shown. DNA preparations were obtained from single mice ($Msh2^{-/-}$, 5 mice; $Ung^{-/-}$, 3 mice), and the number of independent DNA preparations analysed for each gene is indicated in Supplementary Table 2.

indicating that mismatch repair is often unable to repair all of the AID-generated uracils. For genes subject predominantly to error-prone repair in wild-type mice (for example, *Igh*, *Bcl6*, *Cd83*), loss of Ung resulted in a nearly complete loss of transversion mutations at C•G residues, whereas mutation of A•T residues and overall mutation frequency were less affected (Fig. 3 and Supplementary Fig. 7), consistent with the current model for somatic hypermutation[1] (Fig. 2a, inset). However, for *Myc*, at which high-fidelity repair predominates, Ung deficiency yielded a mutation spectrum consisting almost entirely (93%) of C•G transition mutations (Supplementary Fig. 7). Therefore, when Ung is absent, a uracil in the *Myc* locus is left unrepaired or is corrected by mismatch repair. Overall, our data indicate that both Ung and Msh2 contribute to high-fidelity repair of many genes while at the same time acting in an error-prone manner at others. We speculate that this is accomplished by differentially regulating access of error-prone polymerases to these loci (Supplementary Fig. 1).

Genes containing evolutionarily conserved E box motifs CAG (G/C)TG within 2 kb of the transcription start site were significantly enriched among mutated genes in the double knockout ($P < 0.03$) but not wild-type ($P > 0.8$) mutation data sets. This motif forms the core of the binding site for the *E2a*-encoded E12/E47 proteins and its presence in the vicinity of many of the most mutated genes in double knockout B cells (Supplementary Fig. 4) is consistent with the idea that E12 and E47 have a role in recruiting AID to immunoglobulin and non-immunoglobulin loci[10,16,17]. We could not detect a correlation between G-richness and mutation frequency in wild type or double knockout B cells (Supplementary Fig. 8), in contrast to a previous report that analysed a smaller number of genes[18].

Our data demonstrate that AID has the potential to trigger mutations and chromosomal instability[19,20] more broadly than previously thought, with numerous tumour-related genes, including *Myc*, *Pim1*, *Pax5*, *H2afx*, *Ocab*, *Rhoh* and *Ebf1*, now revealed as AID targets in normal B cells. In many of these genes there is a clear correspondence between regions targeted by AID and sites of chromosomal deletions[21] and translocations (Supplementary Fig. 4) in human B cell lymphomas[7]. These findings are of particular significance for understanding the aetiology of events that initiate B cell oncogenesis, such as *Myc*-immunoglobulin locus chromosomal translocations for which it was previously unknown whether the underlying DNA break in *Myc* could be attributed to AID[22]. Our data also demonstrate that the introduction of mutations can be dissociated from the uracil burden generated by AID in a particular gene, arguing that somatic hypermutation is unlikely to occur by inactivation of high-fidelity repair throughout the nucleus of the B cell[23], by recruitment of error-prone repair factors by AID to each of its sites of action[24], or by a global overloading of the capacity of the high-fidelity repair machinery.

A loss of AID target specificity has been suggested to underlie 'aberrant' somatic hypermutation, in which genes undergo somatic hypermutation in B cell tumours but not normal germinal centre B cells[2,7]. By demonstrating that AID is widely mistargeted in normal B cells, our data provide an alternative explanation for this phenomenon: a breakdown of protective high-fidelity repair during the process of B cell transformation. In germinal centre B cells, this protection could be undermined by any agent or environmental factor that perturbs the balance of DNA repair activities, including cellular stress such as hypoxia, which suppresses expression of mismatch repair enzymes[25], and viral infection, which can induce expression of AID[26] and in some cases alter the expression of error-prone polymerases implicated in somatic hypermutation[27,28]. The importance of these protective mechanisms is likely to extend beyond B cells, as infection with *Helicobacter pylori* induces AID expression and mutations in *TP53* in human gastric epithelial cells[29].

METHODS SUMMARY

Mice and cell isolation. $Aid^{+/-}$ mice (C57BL/6 background) and $Msh2^{+/-}$ and $Ung^{+/-}$ mice (mixed C57BL/6xBalb/C background) were used to generate mice of the appropriate genotypes. $B220^+CD19^+GL7^-$ (naive) and $B220^+CD19^+GL7^+$ (germinal centre) Peyer's patch B cells were isolated by cell sorting and genomic DNA was isolated. In some sorts, PNA staining was substituted for GL7.

Amplification and sequencing. Regions to be sequenced were amplified from 30–100 ng of template genomic DNA using Phusion polymerase (Finnzymes) and oligonucleotide primers as described in Methods. Gel-purified fragments were cloned using a Topo TA cloning kit (Invitrogen) and sequenced bidirectionally in 384-well format at the Broad Institute or Agencourt Bioscience Corporation.

Data analysis and statistics. Mutations were scored only at positions passing Neighbourhood Quality Standard (NQS) criteria as described in Methods. Mutation frequencies were calculated as the number of mutations divided by the total number of nucleotides passing the NQS criteria. Statistical comparisons of mutation frequencies were performed using the Fisher's exact test (one tailed) and the significance of AID hotspot enrichment and A>T bias was assessed using the binomial test. Where appropriate, P values were corrected for multiple testing by computing the false-discovery rate[30] with $\lambda = 0$ to generate q values.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature. A summary figure is also included.

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Author Contributions M.L. and D.G.S. designed the experiments and M.L. generated all of the sequencing and expression data. M.L., J.L.D. and S.H.K. performed data and statistical analyses and created the figures. D.J.R., J.L.D. and S.H.K. wrote software for sequence analyses. C.G.V. and C.C.G. provided microarray expression data. D.G.S. wrote the paper. All authors commented on the manuscript.

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LETTERS

The structural basis of protein acetylation by the p300/CBP transcriptional coactivator

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The transcriptional coactivator p300/CBP (CREBBP) is a histone acetyltransferase (HAT) that regulates gene expression by acetylating histones and other transcription factors. Dysregulation of p300/CBP HAT activity contributes to various diseases including cancer[1–4]. Sequence alignments, enzymology experiments and inhibitor studies on p300/CBP have led to contradictory results about its catalytic mechanism and its structural relation to the Gcn5/PCAF and MYST HATs[5–9]. Here we describe a high-resolution X-ray crystal structure of a semi-synthetic heterodimeric p300 HAT domain in complex with a bi-substrate inhibitor, Lys-CoA. This structure shows that p300/CBP is a distant cousin of other structurally characterized HATs, but reveals several novel features that explain the broad substrate specificity and preference for nearby basic residues. Based on this structure and accompanying biochemical data, we propose that p300/CBP uses an unusual 'hit-and-run' (Theorell–Chance) catalytic mechanism that is distinct from other characterized HATs. Several disease-associated mutations can also be readily accounted for by the p300 HAT structure. These studies pave the way for new epigenetic therapies involving modulation of p300/CBP HAT activity.

The p300/CBP protein contains several well-defined protein-interaction domains as well as a centrally located 380-residue HAT domain (Supplementary Fig. 1). To obtain direct information on p300/CBP acetyltransferase structure, enzymatic mechanism and inhibition, we prepared homogeneous p300 HAT domain for high-resolution X-ray structure determination. Because the production of recombinant p300/CBP HAT domain protein in quantities necessary for crystallization was difficult using conventional methods owing to the promiscuous autoacetylation properties of the p300 HAT, predominantly within a modulatory p300 'autoacetylation loop'[10,11], we generated semi-synthetic human p300 HAT domain by expressed protein ligation[10] (Supplementary Fig. 1b). For the p300 HAT preparation, an inactive amino (N)-terminal recombinant fragment was ligated to a synthetic peptide to yield active, minimally acetylated p300 HAT domain. To further aid in crystallization, we genetically deleted 32 residues (1523–1554) of the proteolytically sensitive autoacetylation loop[10], and treated the Lys-CoA inhibitor liganded-p300 HAT domain with trypsin to yield a heterodimeric 28 kilodalton (kDa) N-terminal subdomain (N subdomain) and an 11 kDa carboxy (C)-terminal subdomain (C subdomain) that produced crystals of a p300 HAT–Lys-CoA complex (Supplementary Fig. 1c, d). The structure of the protein–inhibitor complex was determined by a combination of multiple and single anomalous dispersion using selenomethionine- and bromine-derivatized protein and refined to 1.7 Å resolution (Supplementary Table 1).

The overall fold of the p300 HAT domain consists of a central β-sheet comprising seven β-strands surrounded by nine α-helices and several loops, with the last three α-helices and the last β-strand coming from the C subdomain (Fig. 1a and Supplementary Fig. 1d, e).

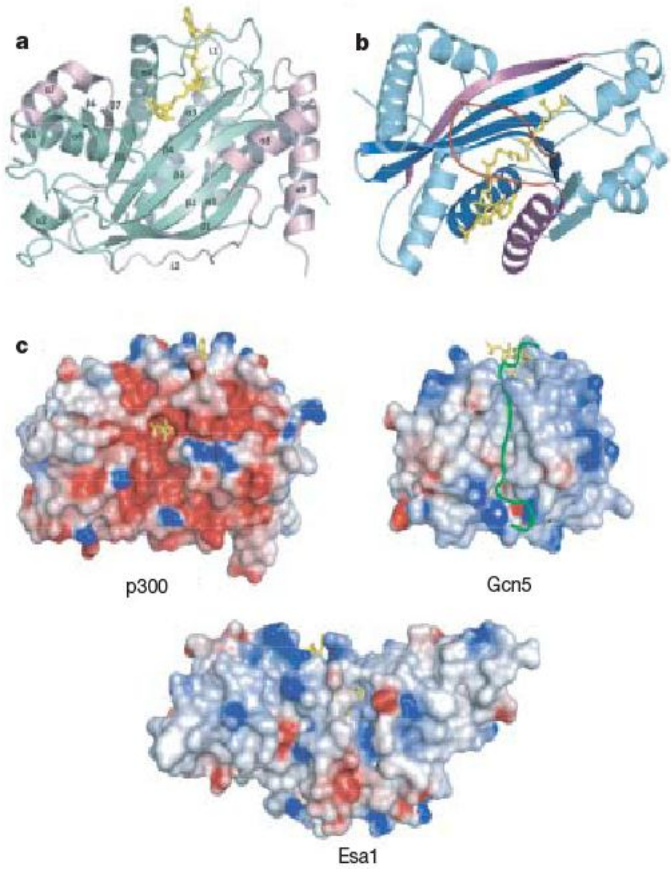

Figure 1 | Structure of p300 HAT domain and comparison of the p300 HAT domain with other HATs. **a**, Overall structure of the p300 HAT domain with N and C subdomains coloured in green and pink, respectively. Lys-CoA is shown in yellow stick figure representation. **b**, p300 HAT domain structure with GNAT structural homology (A, B and D motifs reported by Neuwald and Landsman[13]) highlighted in dark blue and additional homology highlighted in purple. The p300 L1 loop is highlighted in red. **c**, Electrostatic surface representations of the HAT domains of p300, Gcn5 and Esa1. Lys-CoA in the p300 HAT, and CoA in Gcn5 HAT and Esa1 HAT, are represented in yellow stick representation; the histone substrate of Gcn5 HAT is shown in green ribbon. Electrostatic potentials were calculated with GRASP, drawn to the same scale, and imported into PyMOL (http://www.pymol.org) for rendering.

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The smaller C subdomain spans the entire structure by capping opposite ends of the larger N subdomain with secondary structural elements that are connected by a long loop (L2) that tracks along the 'bottom' side of the N subdomain. Another unusually long loop (L1) of the N subdomain is intimately associated with Lys-CoA inhibitor binding. The intimate association of the N and C subdomains is consistent with their protease resistance and tight heterodimeric association during purification and crystallization. A comparison of the p300 HAT domain with other HAT structures[12] shows several differences and some similarities, despite the absence of detectable sequence conservation. Specifically, an overlay of the p300 HAT domain with the HAT domains from yeast Gcn5, a member of the Gcn5/PCAF family of HATs, and from yeast Esa1, a member of the MYST family of HATs, shows structural conservation within the central core region associated with acetyl-CoA cofactor binding[13] (Fig. 1b and Supplementary Fig. 2).

Other aspects of the p300 HAT domain are very different from other HATs. In particular, regions flanking the central core region and the unusually long L1 loop that appears to encapsulate the Lys-CoA inhibitor are unique features of the p300 HAT domain (Fig. 1b). Although the pantetheine arms occupy similar positions with respect to the core domain of all three HATs, the adenosine rings adopt diverse conformations. The adenosine rings in the Gcn5 HAT and Esa1 HAT structures are less ordered compared with their well-ordered counterpart bound to p300 HAT, potentially one of the consequences of the L1 loop interactions with the CoA portion of the inhibitor (Fig. 2a and Supplementary Fig. 2). Indeed, the L1 loop contributes about 30% of the total buried solvent accessible surface of 1225 Å^2 of the CoA portion of Lys-CoA. The L1 loop also appears to be in position to influence protein substrate binding, burying 266 Å^2 of the lysine portion of the Lys-CoA inhibitor. In addition, comparison of the electrostatic surface potential of the substrate-binding surfaces of the HAT domains of Gcn5, Esa1 and p300 shows significant divergence (Fig. 1c). Although Gcn5 and Esa1 show deeper and more apolar substrate-binding pockets, the p300 HAT domain reveals a shallow and highly acidic site, consistent with the different substrate-binding properties of p300 HAT relative to other HATs.

The CoA portion of the Lys-CoA makes extensive interactions with the p300 HAT domain (Fig. 2a, b). In particular, the pantetheine arm of the CoA portion of the inhibitor makes extensive interactions with the p300 L1 loop, which in turn appears to help configure the L1 loop to make extensive intramolecular interactions with other regions of the p300 HAT domain (Fig. 2c). In addition, Arg1410 appears to make several key hydrogen bonds with phosphates of the Lys-CoA molecule, and we have confirmed its importance in 3′-phosphate interaction by mutagenesis and inhibition studies with 3′-dephospho-Lys-CoA[14] (Fig. 2d, Supplementary Figs 3 and 4 and Supplementary Table 2). The lysine component of the Lys-CoA inhibitor also makes extensive interactions with the p300 HAT domain (Fig. 2e). Specifically, the ε-nitrogen atom makes a hydrogen bond with the main chain oxygen of Trp1436; and three hydrophobic residues Tyr1397, Trp1436 and Tyr1446, together with Cys1438, form a hydrophobic tunnel that interacts with the aliphatic portion of the lysine side chain.

In addition to the pocket that accommodates the lysine moiety of Lys-CoA, presumably mimicking the lysine side chain from native protein substrates, a second pronounced and highly electronegative pocket is present about 10 Å away from the substrate lysine (Fig. 3a). Because p300 substrates show a strong preference for the presence of a Lys or Arg side chain three to four residues downstream or upstream of the primary acetylation site (ref. 9) (Fig. 3b), we considered the possibility that this region might be important for the binding of protein substrates. A narrow, shallow and electronegative groove connects these two pockets, with the wall of one side of the groove composed of Ser1396 and Tyr1397 (Fig. 3a). The negative potential of the second pocket is largely formed by the side-chain atoms of residues Thr1357, Glu1505, Asp1625 and Asp1628. We performed single, double, triple and quadruple amino-acid mutagenesis on the mentioned residues. As summarized in Fig. 3c and Supplementary Table 2, these mutants show significantly decreased p300 HAT activity, supporting the importance of this region for peptide substrate binding.

We selected the D1625R/D1628R p300 HAT mutant for further analysis of substrate residue preference. To test the influence of electrostatic interactions on substrate sequence selection, we determined the steady-state kinetic parameters for several synthetic H4 tail peptide analogues (listed in Supplementary Table 3). In H4-15, there are two other basic residues (Lys5 and Lys12) near the target Lys8. Here, we compared substrates containing substitutions of Lys5 and Lys12 as well as the simple substrate Ac-Lys-NH_2 with wild-type and mutant p300 HAT. As shown in Supplementary Table 3, the 22-fold V/K difference between wild-type p300 HAT protein and the D1625R/D1628R mutant for H4-15 substrate drops to about sixfold

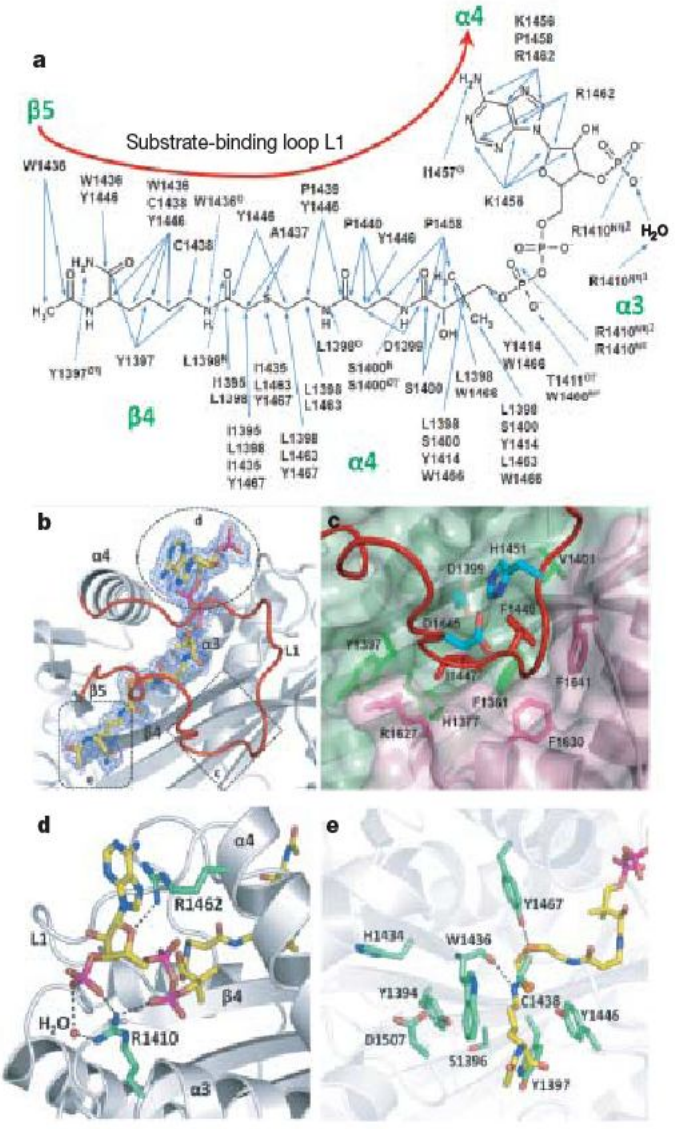

Figure 2 | Lys-CoA recognition by p300. **a**, Schematic views of p300 HAT domain interactions with Lys-CoA. Hydrophobic interactions and hydrogen bonds are indicated with solid and dotted arrows, respectively. **b**, Representative $F_o - F_c$ electron-density omit map contoured at 1.5σ for Lys-CoA; the L1 loop is highlighted in red. Blow-up regions are indicated for panels C–E. **c**, Blow-up view of the tip of the L1 loop. Residues involved in hydrogen-bonding interactions are highlighted in cyan; residues involved in hydrophobic interactions are coloured in red. Green and pink regions of the protein indicate the N and C subdomains, respectively. All hydrogen bonds involving main chain atoms are omitted for clarity. **d**, Blow-up view of interactions stabilizing the ADP–ribose portion of Lys-CoA. **e**, Blow-up view of the lysine acetylation reaction centre with all nearby nucleophilic residues shown in green stick figure.

when using Ac-Lys-NH_2, H4-$12^{K5A/K12A}$ or H4-$12^{K5Cit/K12A}$ as substrates, and less than twofold with H4-$15^{K5D/K12D}$ as substrate. Taken together, these data support the hypothesis that the second highly electronegative pocket (Fig. 3a) binds to a proximal basic residue (Lys or Arg) of the substrate and facilitates the acetyltransferase reaction. Although the lysine-rich autoacetylation loop[10,11] of the p300 HAT domain is not present in our structure, it is interesting to speculate that it may fold back in *cis* onto the highly electronegative surface of the p300 HAT domain that is proximal to the lysine binding site, blocking substrate binding.

To demonstrate catalytic relevance of the crystallized p300 HAT, we showed that the same heterodimeric p300 HAT harbours acetyltransferase activity within threefold of the intact enzyme (Supplementary Fig. 5). The X-ray structure shows several potential nucleophilic residues (Ser/His/Tyr) in proximity to the predicted position of the acetyl-CoA carbonyl carbon (Fig. 2e), but mutagenesis of any of these residues fails to abolish catalytic activity fully (Supplementary Fig. 3 and Supplementary Table 2). Moreover, it has recently been demonstrated that an electrophilic acetyl-coenzyme A affinity labelling-based reagent does not target a residue that is critical for catalysis[15], arguing against a role of an enzyme nucleophile, characteristic of a ping-pong mechanism. However, in contrast to Gcn5/PCAF HAT, which shows an ordered binding ternary

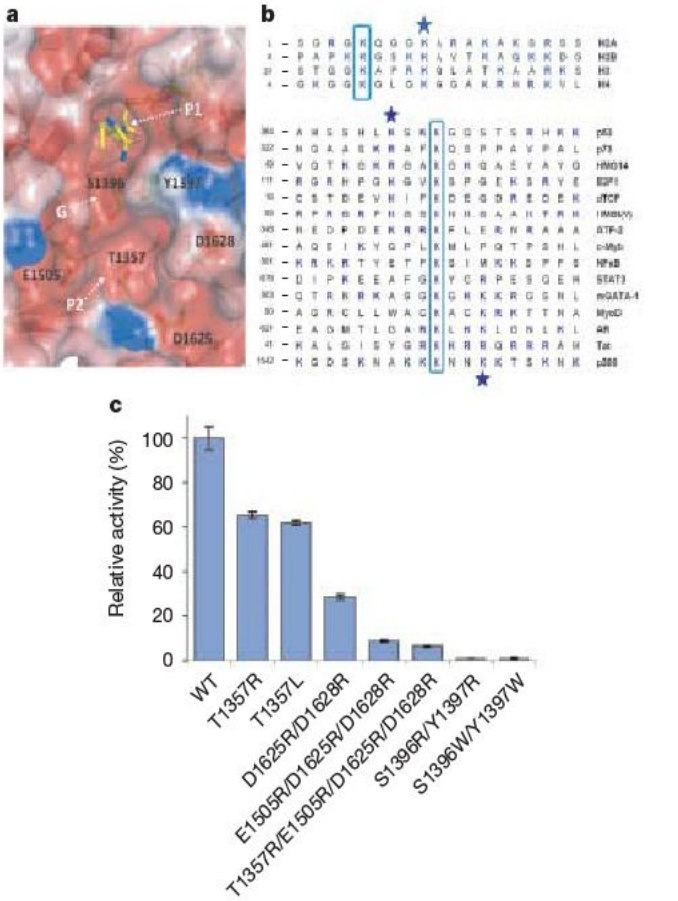

Figure 3 | Substrate-binding surface and mutagenesis studies. a, Blow-up view of the putative substrate-binding site of the p300 HAT domain is shown as an electrostatic surface. Residues contributing to formation of the groove and second pocket are highlighted in stick figure in Corey, Pauling and Koltun (CPK) colouring. Pocket 1, pocket 2 and the connecting groove are annotated as P1, P2 and G, respectively. **b**, Sequence alignments of histone (top) and non-histone (bottom) p300 substrates with all positively charged residues highlighted in blue. The preferred acetylation sites are highlighted in the blue box, and the proximal lysine or arginine residues are marked by a blue star. **c**, Mutagenesis study showing the relative activity of several putative substrate-binding mutants. The activity was measured using 50 nM p300 HAT, 200 μM AcCoA and 200 μM H4-15, and is plotted as activity relative to wild-type p300. Error bars represent spans of duplicates.

complex mechanism, bi-substrate analogues with longer peptide moieties are weaker binders to p300/CBP HAT. For example, H4-CoA-20, a bi-substrate analogue composed of 20 residues of the histone H4 tail, inhibits p300 about 20-fold more weakly than Lys-CoA[8], despite the fact that the same 20-amino-acid histone H4 tail peptide (H4-20) is processed at least 15-fold faster than Ac-Lys-NH_2 (ref. 9) (Supplementary Table 3).

If a classical ordered binding, ternary complex mechanism were operative for p300, then more authentic bi-substrate analogues would be predicted to bind to p300 more tightly than the primitive Lys-CoA analogue, as occurs with Gcn5/PCAF[8]. To rationalize this discrepancy, we hypothesize that a 'hit-and-run' or Theorell–Chance acetyl transfer mechanism[16] could account for such p300 behaviour. In the Theorell–Chance mechanism, there is no stable ternary complex as formed in a standard sequential mechanism (Fig. 4a). Rather, after the acetyl-CoA binds, the peptide substrate associates weakly with the p300 surface, allowing the lysyl residue to snake through the p300 tunnel and react with the acetyl group. We speculate that we have captured the post-reaction state in the structure reported here where peptide-enzyme interactions would be further weakened[7]. Thus, the 'partial' bi-substrate analogue Lys-CoA, rather than the apparently more complete structure H4-CoA-20, is a more faithful mimic of the Theorell–Chance reaction coordinate of p300, and the X-ray structure reveals this reactive state.

We note that the parallel line pattern previously observed in a steady-state two-substrate kinetic analysis of full-length p300 (ref. 9) (and reproduced here with kinetic analysis of the p300 HAT domain (Supplementary Fig. 7c)), suggestive of a ping-pong model[16], is also consistent with a Theorell–Chance mechanism if acetyl-CoA binding is very tight[16]. Although the affinity of p300 for acetyl-CoA, which is highly reactive, cannot be measured directly, here we show that the stable analogue acetonyl-CoA possesses a $K_d = 740$ nM (Supplementary Fig. 6), of sufficient potency to account for a Theorell–Chance mechanism with an apparent parallel line pattern. We have also used a fluorescence assay to show that CoASH binds efficiently to p300 HAT ($K_d = 7.3$ μM, Supplementary Fig. 6), arguing against a ping-pong model[16].

To further assess the p300 HAT kinetic mechanism, we studied product inhibition with Lys-8-acetylated H4-$12^{K5R/K12R}$ peptide and CoASH. These studies reveal that acetylated peptide is competitive versus substrate peptide and non-competitive (mixed-type) versus acetyl-CoA; whereas CoASH is competitive versus acetyl-CoA and non-competitive versus substrate peptide (Fig. 4b, c and Supplementary Figs 7a, b and 8). This inhibitory pattern uniquely supports a Theorell–Chance model rather than a standard ordered binding, ternary complex or ping-pong mechanism[16] (Supplementary Fig. 7a). The broad sequence selectivity of p300/CBP substrates is nicely explained by a Theorell–Chance mechanism that does not involve tight-knit substrate interactions with an extended peptide sequence motif.

The precise role of various residues in facilitating the chemistry of acetyl transfer is suggested by mutagenesis and the observed structure (Fig. 2e and Supplementary Table 2). Of several residues that we mutated within the active site, mutation of Tyr1467 showed the greatest defect, with a Phe substitution showing a 430-fold reduction in V/K. This residue forms a direct hydrogen bond to the sulphur atom of Lys-CoA and is predicted to play a key orienting role as well as general acid function in protonating the leaving group[17]. A second key residue is Trp1436 with an Ala substitution showing a 50-fold reduction in V/K (Supplementary Table 2). The Trp1436 indole side chain appears to play a key role in guiding the Lys side chain into attacking acetyl-CoA by van der Waals contact (Fig. 2e). By contributing to the hydrophobic environment in the active site, it also likely helps reduce the Lys pK_a, biasing the substrate Lys to the neutral amine, which is necessary for attack.

Although the backbone carbonyl of Trp1436 makes an H-bond with the ε-amino hydrogen of Lys-CoA, there is no obvious catalytic

base, in contrast to other HATs where acidic residues are believed to play this role. However, the spontaneous reaction between a neutral amine and thioester is predicted to be very fast once the enzyme can template their association in correct orientation[17]. Roles for polar residues for guiding the protons out of the active site are suggested for His1434 and Tyr1394 and/or Asp1507, which contribute modestly to catalysis (Supplementary Table 2). An analysis of pH-rate profiles in the wild-type reaction (Supplementary Fig. 9) and selected mutants (data not shown) fails to account for the important pK_a of 8.4, which we speculate may correspond to the substrate Lys ε amino group. Based on the discussion above, we present a catalytic mechanism (Fig. 4a).

The structure of p300 HAT allows a more detailed understanding of p300/CBP inactivating mutations that have been observed in various cancers and Rubinstein–Taybi Syndrome[18–21]. Two such residues, Arg 1410 and Trp1436, have been discussed above. Mutations of Gly1375 and Asp1399 are likely to have effects on the conformation of the L1 loop, whereas mutations of Arg1342 and Ser1650 can be rationalized as modulating longer-range interactions in the three-dimensional structure (see Supplementary Information).

Given the therapeutic potential of p300/CBP inhibitors in cancer[4,22–24], cardiac disease[25], diabetes mellitus[26] and HIV[27], the structure reported here should assist in the design of more specific compounds based on the Lys-CoA scaffold. Although the parent compound Lys-CoA shows poor pharmacokinetic properties, more recent strategies for drug delivery[28,29] have largely overcome this limitation. Potentially equally important would be compounds that could complement disease-associated mutants[30] that are associated with p300/CBP HAT inactivation.

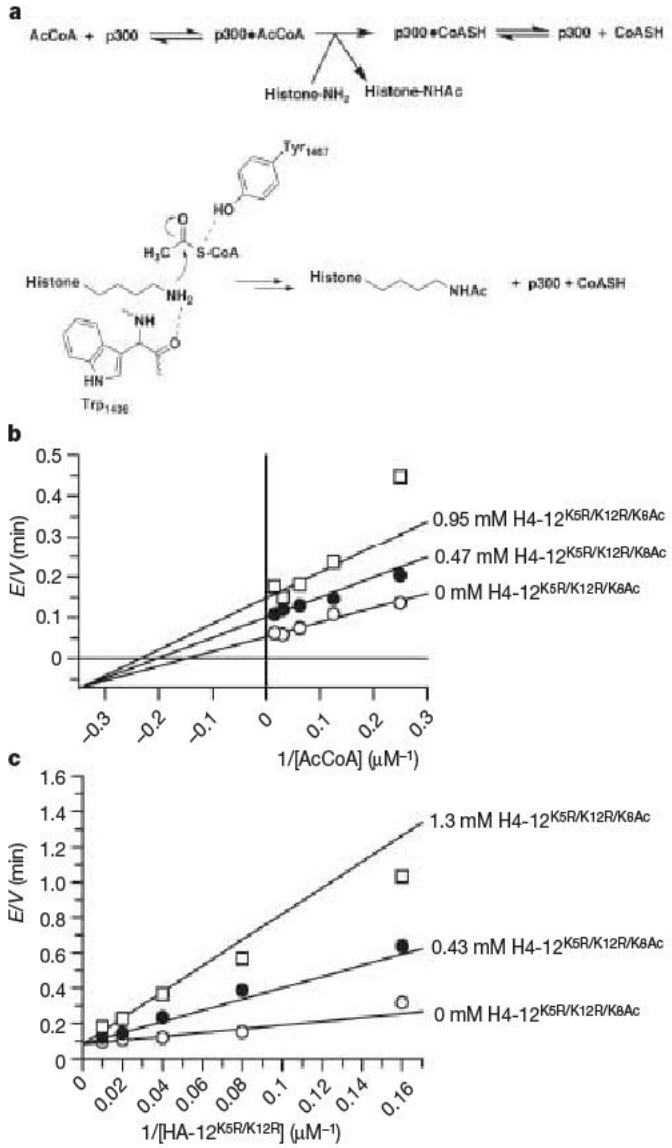

Figure 4 | Catalytic mechanism of p300 HAT. a, Schematic representation of the catalytic Theorell–Chance mechanism of p300/CBP. **b**, E/V versus 1/[AcCoA] at a fixed concentration of H4-$12^{K5R/K12R}$ (20 μM) and variable concentrations of the H4-$12^{K5R/K12R/K8Ac}$ peptides. The p300 HAT concentration = 20 nM. The best fit is to a mixed-type inhibition pattern rather than competitive inhibition based on visual inspection and relative error. The apparent $k_{cat} = 0.32 \pm 0.02\ s^{-1}$, the apparent K_m for AcCoA is 6.7 ± 1.2 μM, the apparent $K_{is} = 1.2 \pm 1.0$ mM and the apparent $K_{ii} = 0.52 \pm 0.09$ mM. **c**, E/V versus 1/[H4-$12^{K5R/K12R}$] at a fixed concentration of AcCoA (20 μM) and variable concentrations of H4-$12^{K5R/K12R/K8Ac}$ peptide. The p300 HAT concentration = 20 nM. The best fit is to a competitive inhibition pattern rather than mixed-type inhibition based on visual inspection and relative error. The apparent $k_{cat} = 0.20 \pm 0.01\ s^{-1}$, the apparent K_m for H4-$12^{K5R/K12R}$ is 12.5 ± 1.8 μM and the apparent $K_{is} = 190 \pm 27$ μM.

METHODS SUMMARY

All peptides were prepared using 9-fluorenyl-methoxycarbonyl solid-phase peptide synthesis as described in Supplementary Information. Semi-synthetic p300 HAT domain was prepared as previously described[10] with some modifications as outlined in Supplementary Information. Briefly, residues 1287–1653 containing a Δ1523–1554 deletion were ligated to residues 1653–1666. The semi-synthetic p300 HAT was purified by cation exchange chromatography and then subjected to limited proteolyisis in the presence of Lys-CoA. The Lys-CoA–p300 HAT heterodimer complex was purified by anion exchange and gel filtration chromatography. The co-purified heterodimer containing N- and C-terminal p300 HAT subdomains bound to Lys-CoA was crystallized in space group $P4_3$, and the structure was determined by multiple anomalous dispersion and refined to a resolution of 1.7 Å as described in Supplementary Information. HAT mutants were prepared by the QuickChange method, and HAT assays were performed by a ^{14}C-acetyl-CoA radioactive[9] assay or a DTNB colorimetric[10] assay as described in Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions X.L. and L.W. designed and performed reported experiments and prepared manuscript figures and text; K.Z. and P.R.T. developed key reagents and performed preliminary studies that led to the reported experiments; Y.H. developed a key reagent and designed and interpreted some of the experiments. R.M. and P.A.C. designed and supervised experiments and prepared manuscript text. All authors read and approved the submitted manuscript.

Author Information The structure of the p300 HAT–Lys-CoA complex has been submitted to the Protein Data Bank under accession number 3BIY. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.M. (marmor@wistar.org) or P.A.C. (pcole@jhmi.edu).

LETTERS

The X-ray crystal structure of RNA polymerase from Archaea

Akira Hirata[1], Brianna J. Klein[1] & Katsuhiko S. Murakami[1]

The transcription apparatus in Archaea can be described as a simplified version of its eukaryotic RNA polymerase (RNAP) II counterpart, comprising an RNAPII-like enzyme as well as two general transcription factors, the TATA-binding protein (TBP) and the eukaryotic TFIIB orthologue TFB[1,2]. It has been widely understood that precise comparisons of cellular RNAP crystal structures could reveal structural elements common to all enzymes and that these insights would be useful in analysing components of each enzyme that enable it to perform domain-specific gene expression. However, the structure of archaeal RNAP has been limited to individual subunits[3,4]. Here we report the first crystal structure of the archaeal RNAP from *Sulfolobus solfataricus* at 3.4 Å resolution, completing the suite of multi-subunit RNAP structures from all three domains of life. We also report the high-resolution (at 1.76 Å) crystal structure of the D/L subcomplex of archaeal RNAP and provide the first experimental evidence of any RNAP possessing an iron–sulphur (Fe–S) cluster, which may play a structural role in a key subunit of RNAP assembly. The striking structural similarity between archaeal RNAP and eukaryotic RNAPII highlights the simpler archaeal RNAP as an ideal model system for dissecting the molecular basis of eukaryotic transcription.

RNAP is the central enzyme of gene expression, and all life forms have RNAPs that function as multi-subunit protein complexes (multi-subunit RNAP) with subunit compositions that vary depending on the domain of life[5]. The common core of the multi-subunit RNAPs comprises five subunits that are conserved from bacteria to humans. Bacterial RNAP is the simplest form of this family (composed of the minimum $\beta'\beta\alpha_I\alpha_{II}\omega$ subunits), whereas in Eukarya, RNAPII possesses additional polypeptides to form a 12-subunit complex, which is responsible for synthesizing all mRNAs in the cell. Previous crystal structures of bacterial core[6] and holoenzymes[7], as well as RNAPII[8–14], have provided insight into structural and functional aspects of RNAPs, but the archaeal RNAP structure remains to be elucidated. On the basis of subunit composition, sequence similarity and the requirement of general transcription factors for promoter recognition and transcription, archaeal and eukaryotic RNAPs have been proposed as being structurally similar. A crystal structure of the archaeal RNAP, however, has been needed to allow a precise comparison of the transcriptional machineries from all three domains of life, and to generate new insights about structural domains and motifs of cellular RNAPs that participate in the assembly of these multi-component molecular machines and in transcription processes.

We have purified native RNAP from *S. solfataricus* for crystallization and structure determination (see Methods). Because of the high sequence conservation among RNAPs from all species in Archaea[15], insights derived from the *S. solfataricus* RNAP can be generalized to the transcription apparatus from this entire domain. The overall structure of the RNAP resembles a 'crab claw' with a protruding 'stalk' formed by the E′/F subcomplex (Fig. 1a). The relative positioning of the RNAP core and stalk are highly conserved between archaeal RNAP and the three classes of eukaryotic RNAPs (Fig. 2)[16]. In the crystal structure of archaeal RNAP, the clamp is in its closed conformation (Supplementary Fig. 1), most probably because of the presence of the E′/F subcomplex. Structural studies of RNAPII have proposed that the function of the stalk (Rpb7/Rpb4 subcomplex) is to modulate the clamp conformation: in the absence of the subcomplex, the RNAPII clamp is in the open conformation, whereas in the presence of the subcomplex, the clamp is closed[8,11,12]. In archaeal RNAP, another function of the E′/F subcomplex has been proposed from *in vitro* reconstitution studies of archaeal RNAP: it facilitates transcription bubble formation of the RNAP–promoter DNA complex under certain conditions[17–19].

The archaeal RNAP structure enables a direct comparison of RNAPs from Bacteria, Archaea and Eukarya (Fig. 2). Conserved structures are found around the vicinity of the active centre and DNA-binding channel (Supplementary Figs 2 and 3) which reflect the fact that the fundamental transcription mechanism is conserved

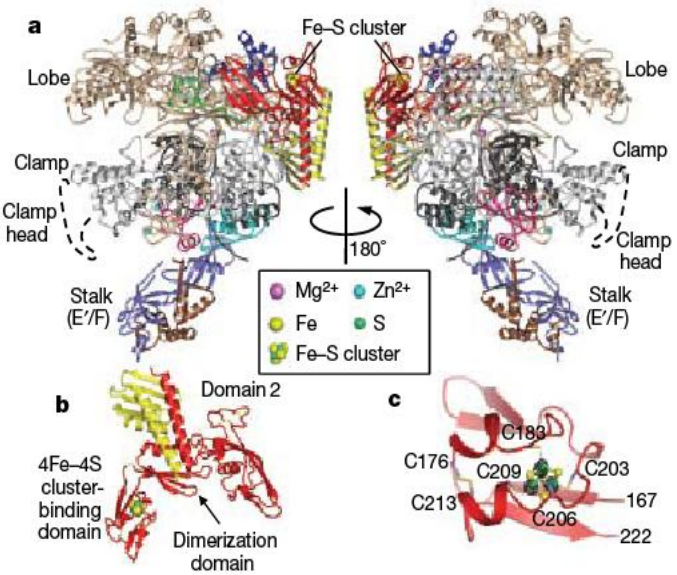

Figure 1 | Three-dimensional structure of the archaeal RNAP. a, Crystal structure (3.4 Å resolution) of the *S. solfataricus* RNAP. Each subunit is denoted by a unique colour (see surface representations in Fig. 2 for colour-code and subunit–subunit interaction). The disordered clamp head domain is indicated as a dotted line. **b**, Crystal structure (1.76 Å resolution) of the D/L subcomplex (red, D subunit; yellow, L subunit). Domain organization is shown. **c**, Close-up view of the 4Fe–4S cluster-binding domain (residues 167–222 of the D subunit). Electron density calculated using the Fe-anomalous signal is shown in black mesh (sigma cutoff = 5).

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among cellular RNAPs. The structures of the surfaces vary between the bacterial and the eukaryotic enzymes (Supplementary Fig. 2b); in contrast, there is striking structural conservation between archaeal and eukaryotic enzymes distributed throughout the entire structure (Supplementary Fig. 2a). For the largest subunit, the entire architecture of Rpb1 (RNAPII) can be superimposed on the A′/A″ subunits (archaeal RNAP) with small deviations around the foot domain (Figs 3a, b and Supplementary Fig. 4b; structural similarity is 68.9%, Supplementary Table 1). Counterparts for the Rpb1 jaw and clamp head domains are disordered in the archaeal RNAP crystal structure, making their structural similarities uncertain. In contrast, the structural similarity between Rpb1 and the bacterial β′ subunit is only 50% (Supplementary Table 1); there are substantial differences in the foot (Fig. 3c), pore1, cleft and dock domains, and there are no Rpb1 jaw and clamp head counterparts in the β′ subunit (Supplementary Fig. 4c). In addition, *Thermus aquaticus* β′ contains a lineage-specific insertion (*Taq* β′ NCD, 283 residues) between conserved regions A and B[6,20].

In Archaea, the largest subunit is split into two polypeptides, A′ and A″, which are encoded by separate genes in an operon[1,2,15]. Sequence alignments reveal that archaeal A′ and A″ correspond to the amino (N)-terminal two-thirds and the carboxy (C)-terminal one-third of the RNAPII Rpb1 subunit, respectively, and that the junction between A′ and A″ is positioned at the Rpb1 'foot' domain[8] (Fig. 3a and Supplementary Fig. 4). The archaeal RNAP foot domain consists of four α helices (Supplementary Fig. 5a), composed of the C terminus of A′ (15 residues) and the N terminus of A″ (62 residues). The corresponding RNAPII foot has a more complex architecture formed by a continuous polypeptide including eight α-helices and two anti-parallel β-strands (Fig. 3b and Supplementary Fig. 5b), but the same four-α-helix architecture, which is found in the archaeal RNAP, is conserved in the centre of the RNAPII foot domain. In contrast, the bacterial foot domain has a completely different architecture (Fig. 3c).

Compared with the largest subunit, the structural similarity between RNAPII Rpb2 and the archaeal B subunit is higher (90%, Supplementary Fig. 6b and Supplementary Table 1). In contrast, the structural similarity between RNAPII and the bacterial β subunit is only 64.9%, and there are deviations in external domains 1 and 2, the hybrid binding and clamp domains, as well as the flap loop/flap-tip helix region (Supplementary Fig. 6c).

Although the two largest conserved subunits provide most of the catalytic functions, their assembly is dependent on the presence of two other conserved subunits, which form a platform for assembly (the D/L[15], Rpb3/Rpb11 (ref. 8) and α_I/α_{II}[6,21,22] in archaeal, eukaryotic and bacterial enzymes, respectively, Fig. 2). Interestingly, phylogenetic analyses[23] and ultraviolet-visible spectra (Supplementary Fig. 7a) suggested that *S. solfataricus* RNAPs may contain an Fe–S cluster in the D subunit. To obtain direct evidence of an Fe–S cluster within RNAP as well as to determine its chemical structure and chelation to the protein, we solved the crystal structure of the *S. solfataricus* D/L subcomplex at 1.76 Å resolution. The D subunit forms a heterodimer with the L subunit (D/L subcomplex), which has an architecture that is almost identical to its eukaryotic (Rpb3/Rpb11 heterodimer) and bacterial (α homodimer) counterparts (Supplementary Fig. 8). The D subunit consists of three domains: a 4Fe–4S cluster-binding domain, domain 2 and a dimerization domain (Fig. 1b). The folding of domain 2 in the D subunit is highly conserved in Rpb3 and α; however, the four Cys residues in the D subunit form two disulphide bonds whereas the four Cys residues in Rpb3 chelate a Zn^{2+} ion. The α subunit has neither a disulphide bond nor a Zn^{2+}-ion-binding motif in domain 2. The 4Fe–4S cluster-binding domain is unique to *S. solfataricus* RNAP because the corresponding region in Rpb3 forms a loosely packed domain (called the 'loop'[8]) and there is no corresponding domain in the bacterial α subunit. In the 4Fe–4S cluster-binding domain, three Cys residues (C183, C203 and C209) provide ligands for the 3Fe–4S cluster (Fig. 1c) and one additional C206 is positioned nearby, suggesting that the cluster may exist as 4Fe–4S *in vivo* (Supplementary Fig. 7c). Two other residues, C176 and C213, in the 4Fe–4S cluster-binding domain form a disulphide bond. Moreover, a strong Fe-anomalous signal was detected in the RNAP crystal within the 4Fe–4S cluster-binding domain (Supplementary Fig. 9b), which further verifies that the RNAP does indeed carry an Fe–S cluster. Although there are many instances where Fe–S clusters provide redox potential for an enzymatic reaction, the Fe–S cluster in the RNAP is located 45 Å from its catalytic centre (Fig. 1a) and therefore is unlikely to be involved in RNAP catalysis. To examine the role of the Fe–S cluster, we constructed a mutant D subunit where the four Cys residues that coordinate the cluster were all

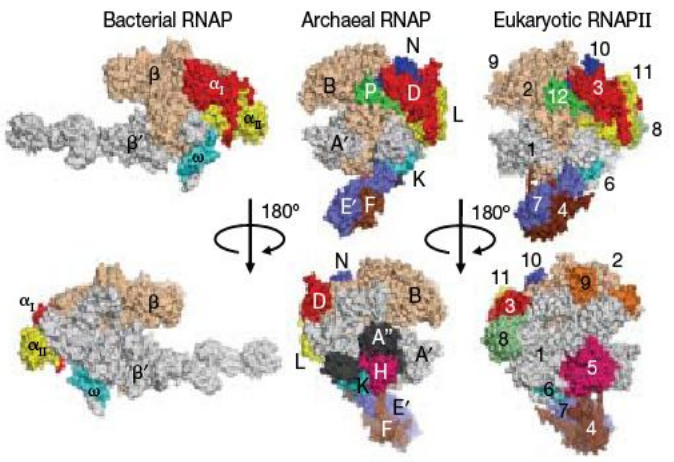

Figure 2 | Cellular RNAP structures from three domains of life. Surface representations of multi-subunit cellular RNAP structures from Bacteria (left, *T. aquaticus* core enzyme[30]), Archaea (centre, *S. solfataricus*) and Eukarya (right, *Saccharomyces cerevisiae* RNAPII[13]). Each subunit is denoted by a unique colour and labelled. Orthologous subunits are depicted by the same colour.

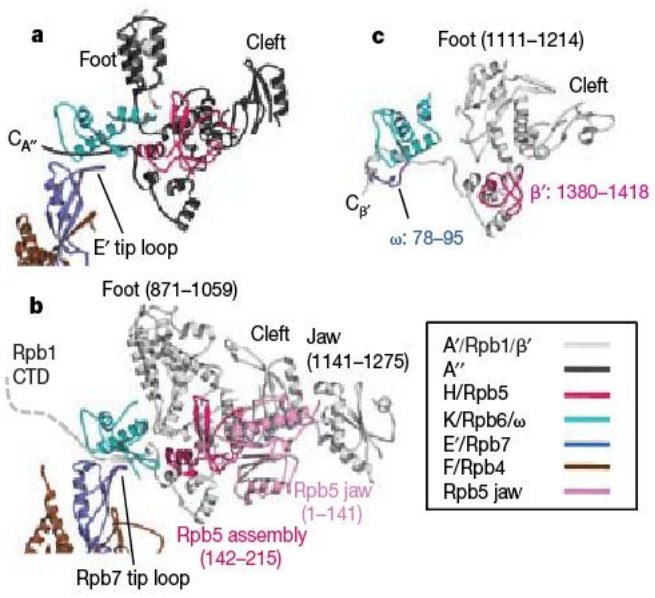

Figure 3 | Structures around the foot domains from three domains of life. **a**, Ribbon model of the archaeal RNAP (the colour coding of each subunit is indicated). Various domains and motifs are labelled. The junction between A′ and A″ is positioned at the foot domain. Subunits H and K associate around the base of the foot. **b**, Ribbon model of the eukaryotic RNAPII[13]. The same four α-helix architecture, which is found in the archaeal RNAP, is conserved in the centre of the RNAPII foot domain. Rpb5 and Rpb6 also associate around the foot domain. **c**, Ribbon model of the bacterial RNAP[30]. The bacterial foot domain has a completely different architecture. The right side of the bacterial foot domain associates with the bacterial-specific insertion of the β′ subunit. In addition, the ω tail wraps around the C terminus of the β′ subunit.

replaced with Ser. The four-Ser variant protein does not chelate the Fe–S cluster, and it forms aggregates even when the D and L subunits are co-expressed in *Escherichia coli* (Fig. 4). We also obtained the same results from D subunit variants having the C183 or C203 residues substituted with Ala (data not shown). Interestingly, the 4Fe–4S cluster-binding domain is not directly involved in heterodimer formation between the D and L subunits (Fig. 1b), but the absence of an Fe–S cluster causes the D subunit to aggregate and prevent the functional D/L subcomplex from being formed. These observations suggest that the cluster may play a role in supporting the structural integrity of the D subunit. When the Fe–S cluster is present within the *S. solfataricus* D subunit, it is not oxygen sensitive because we observed a complete 3Fe–4S cluster in the D/L subcomplex crystal structure, which was purified and crystallized under aerobic conditions. Although the Fe–S cluster can be removed from the D/L subcomplex using the Fe chelator 2, 2′-dipyridyl, it is stable within the assembled RNAP and protected from 2, 2′-dipyridyl treatment (data not shown).

S. solfataricus is not the only archaeon possessing the Fe–S cluster in RNAP (Supplementary Fig. 10). The 4Fe–4S cluster-binding motif has been identified in the RNAP sequence of 16 out of 28 sequenced archaeal genomes, including almost all methanogens (except *Methanocaldococcus jannaschii* and *Methanococcus maripaludis*). In addition, the 4Fe–4S cluster-binding motif is not limited to archaeal RNAPs. Twelve eukaryotic genomes, including those of plants (*Arabidopsis thaliana*) and protozoa (*Tetrahymena thermophila*), encode the 4Fe–4S cluster-binding motif within AC40, the D subunit orthologue of RNAPI and RNAPIII. Furthermore, ultraviolet-visible absorption spectra, as well as iron and acid-labile sulphide analyses, have shown that *A. thaliana* AC40 contains an Fe–S cluster (A.H. and K.S.M., unpublished observations). Our studies show that certain cellular RNAPs are Fe–S proteins. This result raises other intriguing questions, including why only certain cellular RNAPs possess an Fe–S cluster and what is the relation between the presence of an Fe–S cluster in the RNAPs and the environments the cell lives in. The archaeal RNAP structure provides a framework for addressing the question of what functional roles Fe–S clusters play within the transcription machinery of Archaea and Eukarya.

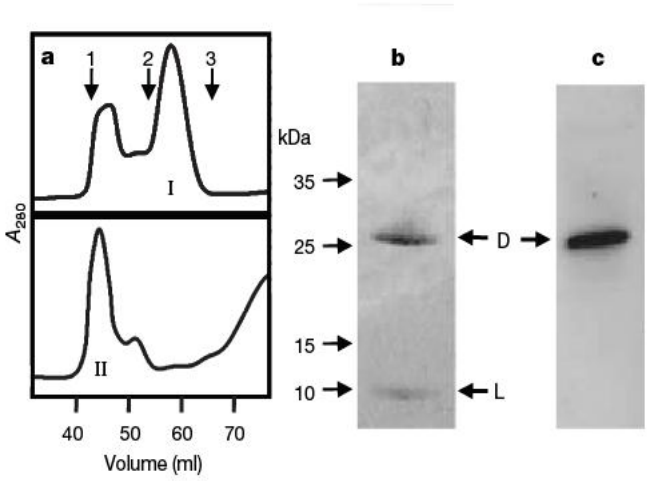

Figure 4 | The Fe–S cluster may play a structural role in D-subunit folding. **a**, Wild-type or variant D subunits were overexpressed in *E. coli* with the L subunit. Partly purified proteins were analysed by gel-filtration column chromatography (top, wild type; bottom, variant). Elution of marker proteins are also shown (1, γ-globulin/158 kDa; 2, ovalbumin/44 kDa; 3, myoglobin/17 kDa). The wild-type D and L subunits formed a heterodimer, which was eluted at approximately 62 ml (**a**, top panel: peak I, mean molecular mass: about 30 kDa, molecular mass of the subcomplex is 40.6 kDa), which was confirmed by Coomassie stained SDS–polyacrylamide gel electrophoresis (**b**). The D subunit variant was eluted at approximately 43 ml (**a**, bottom panel: peak II, mean molecular mass: about 150 kDa) without L subunit that was verified by western blot using an antibody against *S. solfataricus* D/L subcomplex (**c**).

The archaeal K subunit is an orthologue of eukaryotic Rpb6 and bacterial ω subunits, and their folds are highly conserved in the central regions. In archaeal RNAP, subunit K, along with the E′ subunit tip loop, participates in protecting the C-terminal tail of the largest subunit (Fig. 3a), and the arrangement of these subunits is conserved in RNAPII (Fig. 3b). The C-terminal tail (residues 78–95) of the ω subunit, however, has a different trajectory compared with K and Rpb6 (Fig. 3c). The ω tail wraps around and completely covers the C-terminal end of β′. Intriguingly, this bacteria-specific protein fold may have a similar function to the corresponding tip loops of E′ of archaeal RNAP and Rpb7 of RNAPII.

Precise structural comparisons between archaeal and eukaryotic RNAPs reveal structural elements common to both systems; these insights will be useful in elucidating components unique to RNAPII that enable it to perform highly regulated gene expression[24]. The structural differences between these RNAPs are limited to the side of the face (Fig. 2 and Supplementary Fig. 11) that faces downstream DNA in the transcription elongation complex. From a structural perspective, it is interesting that almost all these differences can be classified as simple additions of RNAPII-specific polypeptides, including subunits (Rpb8 and Rpb9) and domains (Rpb1 CTD and Rpb5 jaw) to the archaeal RNAP, rather than changes to the core structure (Supplementary Figs 11 and 12).

One of the keys to understanding the mechanism of an enzymatic reaction is to have a simple and robust system that can be probed *in vitro*. Studies on bacterial transcription are the most advanced because the RNAP can be assembled from recombinant[25] subunits with full activity. This system allows one to use straightforward molecular biology techniques, as well as site-specific incorporation of chemical probes[26,27]. The latter has led to several successful biophysical and biochemical studies on RNAP. Currently, these tools are not applied to eukaryotic RNAPII because this enzyme cannot be reconstituted *in vitro*. Furthermore, because of substantial structural differences in key regions, including the downstream DNA configuration and RNA exit channel[28], the bacterial RNAP may not serve as a complete and representative model system for transcription in eukaryotes. Many biochemical studies, as well as this current crystallographic study, have established that the structural and functional similarities between archaeal RNAP and eukaryotic RNAPII exist on many levels. Furthermore, active archaeal RNAP can be conveniently reconstituted from its individual subunits *in vitro*[17,18,29], and studies of promoter-dependent transcription can easily be established with fully purified general transcription factors and *in vitro* reconstituted RNAP. The crystal structure presented here reveals that archaeal RNAP is not only a simplified version of its eukaryotic RNAPII counterpart but also an excellent model system for dissecting the molecular basis of eukaryotic transcription.

METHODS SUMMARY

***S. solfataricus* RNA polymerase purification, crystallization and structure determination.** Native RNAP was purified from *S. solfataricus* P2 cells for crystallization and structure determination. Primitive monoclinic $P2_1$ crystal contained two 377 kilodalton (kDa) RNAP molecules per asymmetric unit. The final R-work and R-free factors are 27.0% and 34.3%, respectively. Figures were prepared by PyMOL.

Purification, crystallization and structure determination of the *S. solfataricus* D/L subcomplex. An electron-density map at 1.76 Å resolution (Supplementary Fig. 9c) was obtained by a combination of molecular replacement by using the yeast Rpb3/Rpb11 structure[8] as a search model and density modification. The final R-work and R-free factors are 21.0% and 24.7%, respectively.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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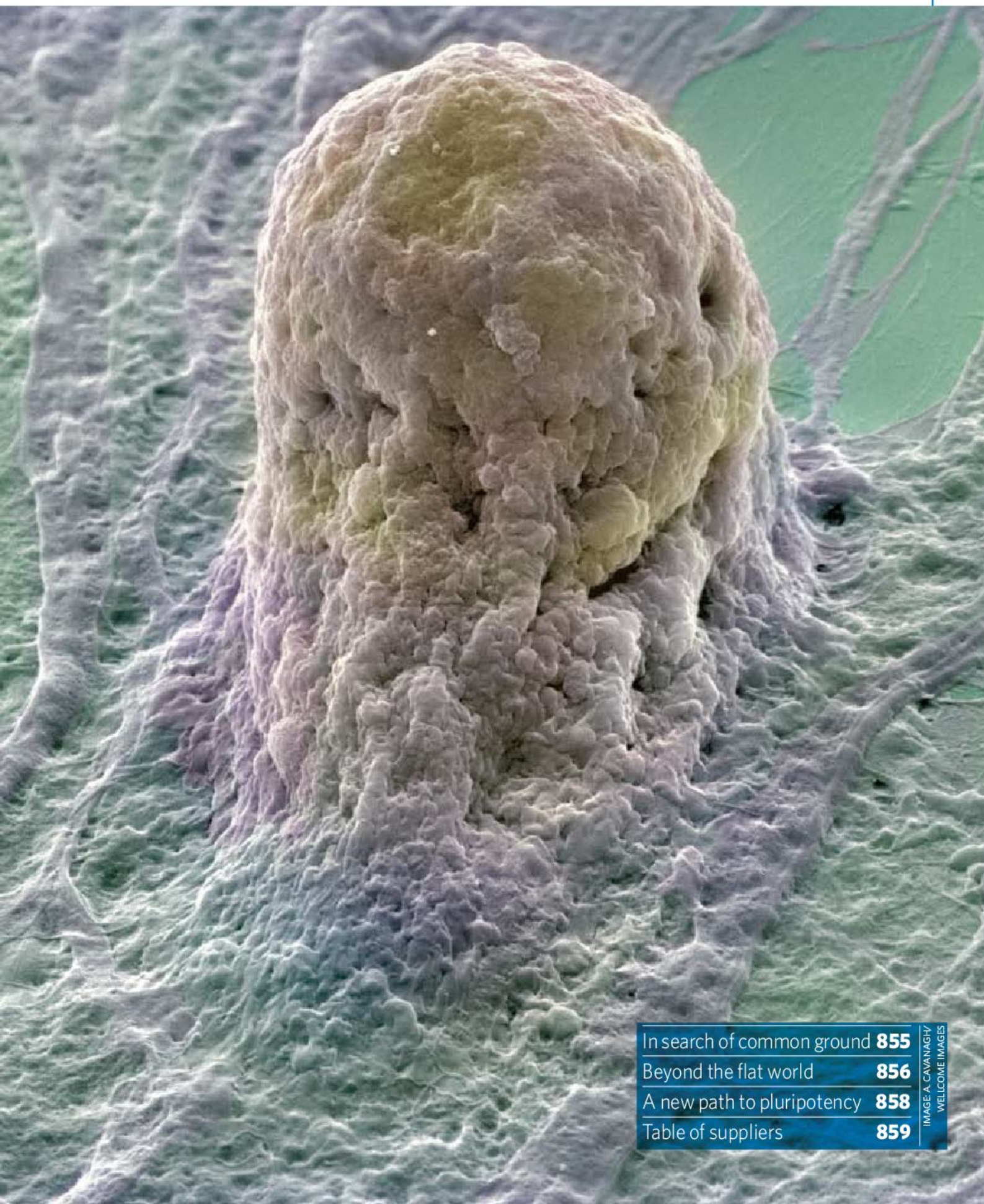
TECHNOLOGY FEATURE
STEM CELLS



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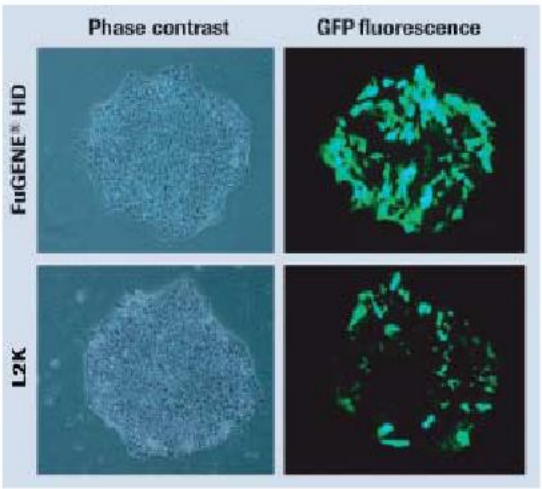

Figure 1: Cell morphology and GFP fluorescence from a transfected gene in human ES cell colonies. Cells were transfected with either FuGENE® HD Transfection Reagent or a transfection reagent from another supplier (L2K). After 24 hours, changes in morphology were not observed when FuGENE® HD Transfection Reagent was used. Furthermore, the percentage of GFP-positive cells was high, and strongly fluorescing cells were observed also in the central area of the colonies.

Data courtesy of Kouichi Hasegawa, Kyoto University, *Biochemica* **4**, 24-26 (2006).

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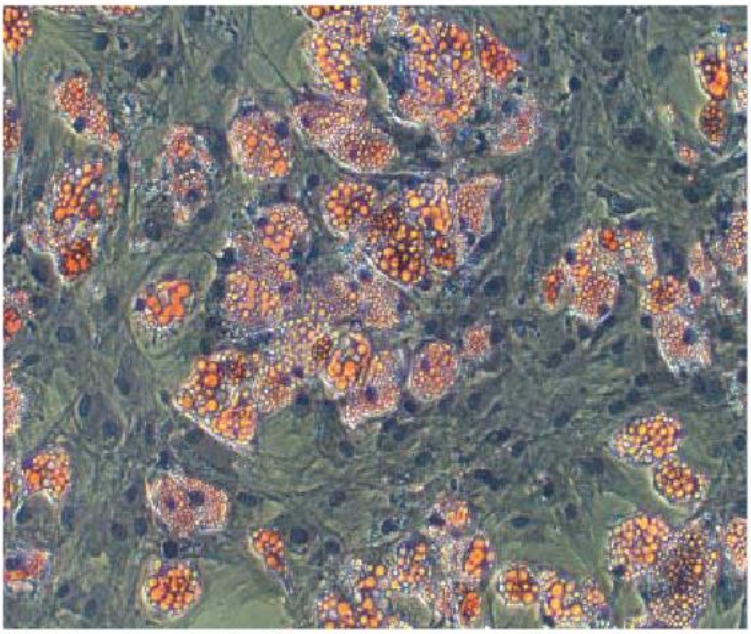
In search of common ground

With the number of stem-cell lines rapidly increasing, technology developers are working to improve systems for culturing and efficient differentiation — all with an eye on the clinic. Nathan Blow reports.

The explosion in stem-cell research that followed the isolation of human embryonic stem cells[1] in 1998 has seen the number of cell lines available to researchers increase dramatically. This burst may be due to the fact that embryonic stem cells are pluripotent — having the potential to generate all adult and embryonic cell types — so there are exceptional possibilities for their use in medicine.

Since 1998, about 200 embryonic stem-cell lines have been derived along with many more adult stem-cell lines. Supporting all these stem cells are many ways to help propagation and differentiation. So it should come as no surprise to learn that there are no standard culture conditions for stem cells. "People sometimes bristle at the word standard," says Derek Hei, director of the US National Stem Cell Bank (NSCB) in Madison, Wisconsin. "In the stem-cell community there is not really a standard culture method."



Adult stem cells undergoing differentiation to adipogenic (fat producing) cells.

THERMO FISHER SCIENTIFIC

But 'standard' should not be an alarming word. "In talking to stem-cell researchers, we found that they are really looking for standardization of culture methods," says Tori Richmond, strategic initiatives specialist at Thermo Fisher Scientific in Waltham, Massachusetts. Arriving at any such standard would require evaluation of all available cell lines on every culturing system — a gargantuan task. But Martin Pera, director of the Institute for Stem Cell and Regenerative Medicine of the University of Southern California in Los Angeles, suspects that larger groups, such as the International Stem Cell Initiative, might tackle the standardization issue in the near future. "I am hoping that within the next couple of years we will arrive at one or two cell-culture platforms that everyone can use."

The cell in a haystack

Several companies and stem-cell banks are now providing a range of older and more recently derived embryonic stem-cell lines to researchers, which has provided a greater understanding of the properties of these cells.

Ethical concerns regarding the use of embryonic stem cells led the US government to establish a list of 21 embryonic stem-cell lines that can be used for research funded by federal sources. To this end, the NSCB was established in 2005 to characterize, hold and distribute these approved human embryonic stem-cell lines. For each of these lines, the bank is currently performing extensive testing, says Hei, including characterization of gene expression profiles, karyotype stability, and other standard embryonic stem-cell assays such as flow cytometry for specific cellular markers of pluripotency.

At present the NSCB offers 15 of the 21 federally approved embryonic stem-cell lines to researchers. "We have set up a website and created master cell banks for each of the lines," Hei says, adding that not only is the characterization data posted online for interested researchers, but the NSCB also provides protocols for propagating and maintaining cell lines on its site. The University of Massachusetts Medical School in Shrewsbury is also expected to open a stem-cell bank to distribute embryonic stem-cell lines derived by researchers within the state, including more than 30 embryonic stem-cell lines to be supplied by Harvard University, during the next year.

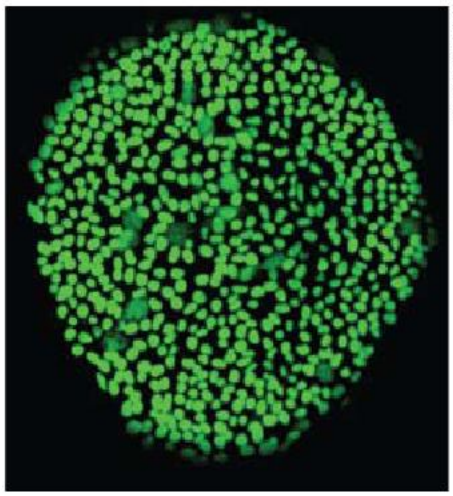
Similar repositories also exist outside the United States. For example, the UK Stem Cell Bank in Hertfordshire, started in 2003, and currently provides eight different human embryonic stem-cell lines. Other new stem-cell banks are on the way.

ES Cell International (ESI) in Singapore is one of a number of commercial sources for researchers looking to obtain human embryonic stem cells. It offers six of the US-approved lines, as well as several other human embryonic stem-cells lines derived at the company. Cellartis in Gothenburg, Sweden, and Millipore in Billerica, Massachusetts, also provide cell lines. Cellartis is one of the largest sources for defined embryonic stem-cell lines, boasting 30 different lines that can be obtained at various stages of passage or as subclones of selected lines. Millipore now offers two human embryonic stem-cell lines, human neural progenitors, as well as several mouse embryonic stem-cell lines.

Search and you will find

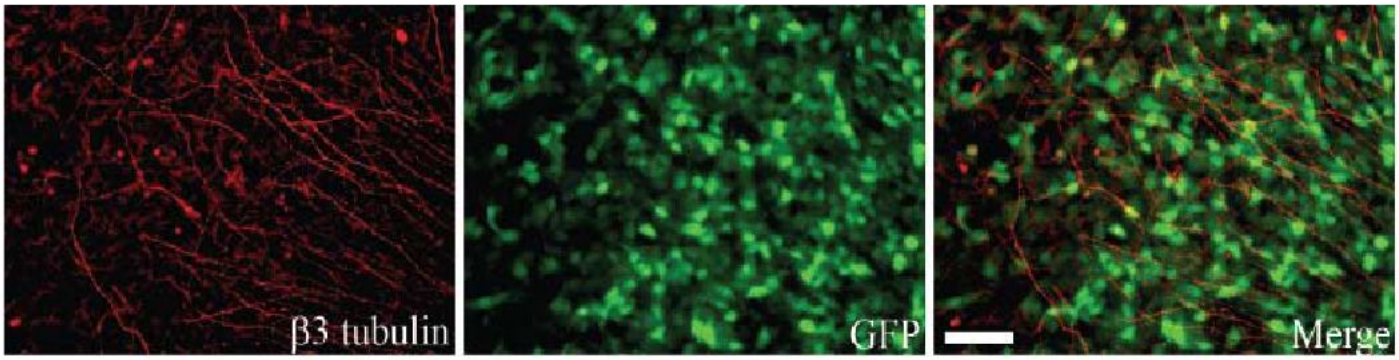
Other companies deriving new human embryonic stem-cell lines can also provide them to researchers. "If people request them, then yes, we will provide lines," says Robert Lanza, chief scientific officer at Advanced Cell Technology, in Worcester, Massachusetts. He says that Advanced Cell Technology also plans to give several lines to the Massachusetts stem-cell bank.

Having many different cell lines available for research might be critical to understanding the true potential of these cells. "It is funny, people say 'embryonic stem cells' but the cell lines all have their own behaviour," notes Lanza. Although a recent study by the International Stem Cell Initiative examining 59 human embryonic stem-cell lines found a high degree of phenotypic similarity between all lines[2], some researchers have noted differences is the behaviour of the cell lines in



BD BIOSCIENCES

Immunochemical staining of undifferentiated human embryonic stem cells cultured on BD Matrigel Matrix in mTeSR -1 medium.



INVITROGEN

Engineered stem cells differentiated into ß3 tubulin-expressing neurons (red) while continuing to express green fluorescent protein.

culture. "Cell lines vary in how easy they are to propagate *in vitro*," says Pera. And Lanza notes that researchers at Advanced Cell Technology can tell embryonic stem-cell lines apart based on the behaviour of the cells in culture.

The mature way to look at stem cells

Although embryonic stem cells are pluripotent, adult stem cells are multipotent, and can only regenerate specific adult cell types in the body. And for those researchers interested in investigating the potential of adult somatic stem cells, several companies are advancing the idea of providing adult stem cells that have been qualified for culturing on specific media.

Thermo Fisher Scientific supplied media and reagents for culturing adult stem cells, but has now moved into supplying adult stem-cells lines as well. "Our strategy has not only been to create kits to support culturing and differentiation of adult stem cells, but also offer the validated stem cells themselves," says Alain Fairbank, research market manager at Thermo Fisher Scientific. The company now offers four mesenchymal (multipotent stromal cells) stem-cell lines, a haematopoietic (germinal cells from umbilical cord blood) stem-cell line, and a recently discovered multipotent cord-blood cell line that Fairbank says seems to be less restricted in its multi-lineage potential. The advantage of this approach is that all media, reagents and cultureware are certified and validated to work with these specific cell lines. "Our focus has been developing tools that are validated to work together with stem cells," Fairbank says.

Invitrogen of Carlsbad, California, is also "heavily focused on mesenchymal research", says Joydeep Goswami, the company's vice-president of stem-cell research. "These cells are probably going to be the first non-haematopoietic stem cells to be used for treating patients." In addition to providing adult stem-cell lines, Invitrogen is also actively researching engineered stem cells. "We have been doing a lot of research to convert stem cells themselves into tools," says Goswami. He says that these engineered cell lines act as single-cell reporters providing a visual readout from a live stem cell as it differentiates to separate lineages.

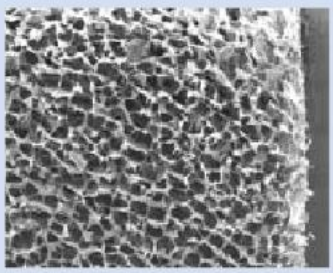
But even as certain adult stem cells become more readily available with well-validated approaches to culturing and differentiation, there is still much work to be done. "Many adult stem-cell populations remain difficult to propagate and expand *ex vivo*," says Pera. And with the exception of mesenchymal stem cells, he says, this has not changed dramatically in recent years. Although Goswami agrees, he also points to another potential issue. "It is not that adult stem cells have not been isolated, the bigger issue is how do you get well characterized stem cells?" He notes that in some instances researchers will call

BEYOND THE FLAT WORLD

In culture, stem cells rely on signals to differentiate to other cell lineages. Although certain growth factors are known to promote some differentiation programmes, it is now becoming clear that physical interactions between cells and mechanical sensing may also help to promote differentiation. So researchers are developing a variety of three-dimensional (3D) matrices for stem-cell differentiation.

In 2006, Dennis Discher of the University of Pennsylvania in Philadelphia, and his colleagues, demonstrated the potential of the matrix alone to promote differentiation. "The idea was to use synthetic gels with a collagen monolayer to mimic the elasticities of certain tissues and see how the cells respond," says Discher. The work was done under constant serum conditions, without any discriminating soluble factors or growth factors that might promote differentiation in one direction or another. The results showed that by simply varying the elasticity of the matrix, the attached mesenchymal stem cells could undergo either neurogenesis, myogeneis or osteogenesis. They went on to show that once the cells adhere, they begin setting up the stress fibres that actively pull on the adhesions and on the matrix outside. "We showed that the cells feel the matrix and respond to it," says Discher.

Stefan Przyborski is the founder of Reinnervate in Durham, UK, the developer of a new scaffold for routine 3D cell culture. "We create ways of making the *in vitro* environment a more realistic environment for cell growth," he says. Reinnervate's scaffold is unique, but might seem familiar to biologists who have performed cell culture. "We created a scaffold made of polystyrene, the same material that people currently



INVITROGEN

Three-dimensional matrices can promote cell differentiation.

grow their cells on." This is important because researchers know how cells respond to this material in two-dimensional applications. But he also notes that this 3D environment enhances differentiation when compared with cells cultured on two-dimensional plates.

Invitrogen of Carlsbad, California has developed Algimatrix, an inert 3D scaffold made of alginate. "The idea behind Algimatrix is that it provides a framework for cells to reside in that will enable formation of spheroidal structures in a controlled manner," says Mark Powers, a director at Invitrogen. Powers says that when embryonic stem cells form embryoid bodies, they can aggregate into very large structures where the cells on the inside can be oxygen or nutrient limited. But with Algimatrix the aggregates grow to a consistent size and not beyond the size of the pores provided by the scaffold. And since it is an inert scaffold, it actually promotes cell interactions. "A scaffold such as Algimatrix would allow a researcher to culture cells in 3D aggregates to promote differentiation."

It is becoming clear that mechanical interaction has a role in cell differentiation. "What we are really describing is a sense of touch — the cells have no eyes or ears, so they use this sense of touch to tell where they are — this is all part of sensing and responding to the environment," says Discher. N.B.

cells a particular type of stem cell, but the next batch will have different marker profiles, indicating a different cell type.

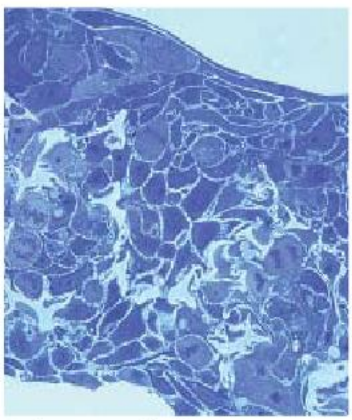
Mouseless developments

The culturing of embryonic stem cells has often relied on the use of mouse embryonic fibroblast cells (MEFs) as 'feeder' cells in different media formulations containing serum. It is thought that the MEFs secrete factors that promote the growth of embryonic stem cells in culture. But growing embryonic stem cells using MEFs in undefined media and serum that potentially contains animal products could limit the potential of stem-cell lines for therapeutic applications.

"It all comes down to how the regulators look at these lines and how they have been handled over the years," says Bruce Davidson, chief scientific officer at ESI. It is for this reason that ESI worked to derive clinical-grade human embryonic stem cells. "We have four GMP- [good manufacturing practice] certified embryonic stem-cell lines that were developed over the past couple of years and are now ready for distribution," says Davidson. Cellartis also provides a human embryonic stem-cell line that was derived without any contact with animal products. And many more researchers and companies are trying to identify the important factors for culturing embryonic stem cells and develop robust feeder-free approaches in well-defined media.

The WiCell Research Institute in Madison, Wisconsin, has a major focus on optimizing embryonic stem-cell culture media and conditions to allow long-term culture of stem cells that do not differentiate. In recent years, researchers at WiCell have found that small changes to either media or the physiochemical conditions of the culture can have a profound effect on the viability of cells. But in 2006, WiCell researchers reported culturing embryonic stem cells without using MEFs, instead relying on protein components that were either recombinantly derived or purified from human materials[3].

R. CARNACHAN



Poylstyrene scaffolds can support a variety of cells in culture.

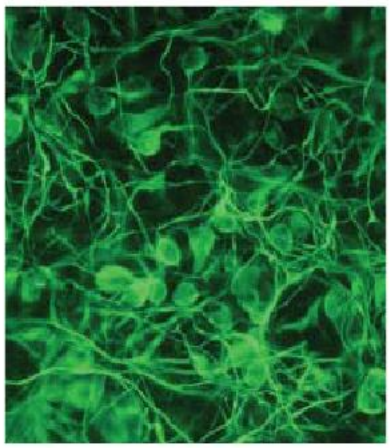
That medium — mTeSR-1 — is now marketed by StemCell Technologies in Vancouver, Canada, who is collaborating with BD Biosciences in San Jose, California, and WiCell on feeder-free cell culture environments for human embryonic stem-cell culture using BD Biosciences Matrigel scaffold. "Colonies formed on Matrigel hESC-qualified matrix with mTeSR-1 media tend to be spread out and form a monolayer-like morphology, which makes passage easier and transfection more efficient," says Marshall Kosovsky, technical support manager at BD Biosciences. Unlike three-dimensional growth matrices, where cell–cell interaction can promote differentiation, Matrigel is coated as a two-dimensional scaffold and is therefore effective for long-term embryonic stem-cell culture. Although Matrigel is a solubilized preparation extracted from a mouse sarcoma — potentially raising similar issues to the use of serum — Kosovsky says that they are currently working on more defined formulations of the scaffold.

Invitrogen is one of several companies moving away from culture conditions that rely on either feeder-cells or serum. "We are providing stem-cell media with a twist — it is a serum-free and defined media," says Goswami. This medium, called StemPro hESC SFM, works with 16 embryonic stem-cell lines from around the world. Millipore also offers a serum-free medium called HEScGRO, which works with a variety of embryonic stem-cell lines.

Different paths

"The field is moving towards defined media without animal components and without feeders," says Pera. But some scientists are being cautious about these culture systems. Although signs are encouraging, Pera says that the jury is still out on most of these new systems. And the NSCB is taking a careful approach to feeder-free cultures. "We made the decision not to use some of the feeder-free methods that are coming out right now, but to stick at this stage to the standard MEF-based methods," says Hei. The decision was made in part to avoid forcing the research community towards feeder-free methods while they are still in the early stages of development. But Hei also acknowledges that at a later date they might develop banks of cells using feeder-free systems. Although some express concerns that cells cultured in feeder-free systems do not thrive as well, Goswami is confident in these new systems and says that the time has come for feeder-free, serum-free embryonic stem-cell culture. "Using our media we can get equivalent or higher numbers of cells" compared with any feeder-dependent system, he says.

"We are still learning a lot about what it takes to coax a stem cell to become a particular tissue," says George Daley from Harvard Medical School in Boston, Massachusetts.



V. CHRISTIE

Reinnervate in Durham, UK, derives neurons from human pluripotent stem cells using synthetic compounds.

Differentiation of embryonic-stem cells or adult stem cells into particular lineages is an area of intense research for developmental biologists and researchers interested in using stem calls for therapeutic applications.

"This is where the battlefront is," says Lanza, "We need to learn how to generate different cell types." He points to haematopoetic stem cells, which give rise to all blood cell types, as an example of how difficult the task can be. In an embryo these cells start out in the yolk sac, then travel to the aortic arch, to the liver and then to the bone marrow, and at each point along that journey are being 'educated' by their surroundings. Replicating this is not easy. Advanced Cell Technology has been working on the differentiation potential of the haemangioblast — a precursor to hematopoietic cells — that they isolated. "People have got other haemangioblast-like cell types, but these are KDR-negative and CD31-negative, so they are an earlier progenitor cell than what's been described previously, which is why they expand and do things so much better," says Lanza. Differentiating these haemangioblasts has allowed the company's researchers to generate entire tubes of red blood cells, as well as other hematopoietic lineages and endothelial cells at very high efficiencies.

Although success has also been reported in using differentiation protocols for deriving neural progenitor cells, cardiomyocytes and retinal pigment epithelium from embryonic stem cells, in most cases studies have shown that only a very small percentage of cells differentiate into a particular tissue type. Some researchers now think that multiple cells or interactions between cells and surfaces might be required to obtain a desired derivative from stem cells (see 'Beyond the flat world').

But Pera sees this as one area that is steadily progressing. "I think we are getting more and better differentiation protocols that are more defined and have better endpoints," he says. However, he does think that there is lack of comparative data on different cell lines and their abilities to differentiate using the current protocols — although the available data would indicate that there will be differences between certain cell lines in the ability to perform in specific differentiation protocols.

Looking up

The world might soon be slightly easier for researchers working on mesenchymal stem-cell differentiation. Both Thermo Fisher Scientific and Invitrogen are introducing kits in the coming months for the differentiation of

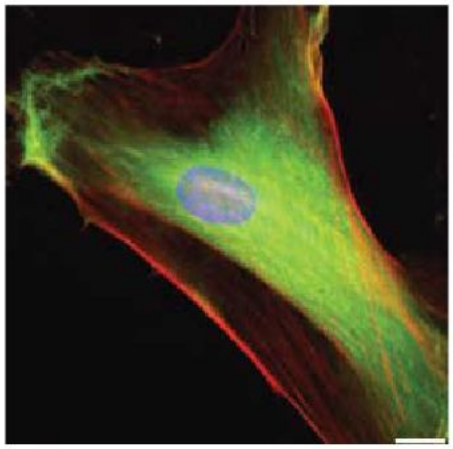
mesenchymal stem cells to adipogenic (fat-producing), osteogenic (bone-producing) and chondrogenic (cartilage-producing) lineages. These kits provide all the necessary growth factors to direct the differentiation of the mesenchymal stem cells. Millipore also offers differentiation media and kits for neural stem-cell lines and mesenchymal stem cells including a human neuronal-differentiation kit and adipogenesis and osteogenesis kits. Although these kits are available for mesenchymal stem cells, the percentages of differentiated mesenchymal cells can still be low for certain lineages.

Stem cells hitting the clinic?

Even as culture and differentiation research progresses, the race is starting to bring embryonic stem-cell therapies to the clinic. Novocell of San Diego, California, is searching for a diabetes treatment, exploring the potential of using stem cells for treatment. "Using cadaveric or even fetal cells was a real challenge because you do not have an unlimited source of these cells," says Alan Lewis, chief executive of Novocell. "While developing a delivery system, in the background we were working with embryonic stem cells to derive insulin-producing cells," he says. And that work is starting to pay off because Novocell can derive definitive endoderm from embryonic stem cells and then differentiate these to insulin-producing cells. Novocell is now working to define these insulin-producing cells, while further refining its polyethylene-glycol or PEG-based delivery vehicle that could help to avoid immune-response issues in patients.

As companies move their products closer to clinical trials, the questions on everyone's mind are when and under what conditions will the US Food and Drug Administration (FDA) fire the starter's gun? Advanced Cell Technology and Geron in Menlo Park, California, are currently in discussions with the FDA regarding clinical trials using embryonic stem cell-based therapies for the coming year.

Advanced Cell Technology has developed an application to use embryonic stem-cell-derived retinal pigment epithelium to treat various retinal degenerative diseases such as macular degeneration and retinitis pigmentosa. The company started with this application because in addition to being able to generate large numbers of the required cells, the eye is an immune privilege site, lessening the possibility of an immune response to the cells. Geron has developed a stem cell-based treatment for acute spinal-cord injury. For both of these applications, the companies have utilized stem cells grown with mouse feeder cells. For Advanced Cell Technology this decision was based on how well the stem cells thrived in culture. "It turns out that although you can derive cells on human feeders or even feeder-free with extracellular matrices, they do not thrive as well," says Lanza. And for this reason he thinks that in the long term, when generating large batches of cells for clinical applications, the mouse feeders are currently the optimal method.



THERMO FISHER SCIENTIFIC

Umbilicial matters: Thermo Fisher Scientific offers adult stem-cell lines derived from Wharton's Jelly.

It is not yet clear what the first therapy based on embryonic stem cells to reach clinical trials will be, whether it will be using cells raised on MEFs or feeder-free media. And for some researchers it is not even clear if embryonic stem cells themselves will be the first pluripotent cells first to reach the clinics (see 'A new path to pluripotency'). George Daley says that it is too early to tell for sure the way stem cell-based therapies will enter the clinics, but he will be watching the developments closely. "I think that it is going to be an exciting time to wait and see," he says. ■

Nathan Blow is the technology editor for *Nature* and *Nature Methods*.

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2. Adewumi, O. *et al. Nature Biotechnol.* **25,** 803–816 (2007).
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A NEW PATH TO PLURIPOTENCY

In November 2007, two groups headed by James Thompson at the University of Wisconsin-Madison and Shinya Yamanaka at Kyoto University in Japan made headlines when they described methods to reprogramme adult human cells to a pluripotent state. These cells, called induced pluripotent stem (iPS) cells, are genetically modified by the integration of up to four DNA-transcription factors into the adult cell genome. Soon after, in December, George Daley, of Harvard Medical School in Boston, Massachusetts, and his colleagues also demonstrated iPS cells could be generated from a wide variety of adult cells. "For any patient we can use the technique and take a skin biopsy to establish a pluripotent cell," says Daley. One of the most valuable aspects of the iPS-cell technology currently, he says, is the ability to perform disease modelling. And to take advantage of this, Daley and his colleagues are working on generating large numbers of disease specific iPS-cell lines.

George Daley is exploring the potential of induced pluripotent cells.

Although an incredible step forward in stem-cell research, iPS cells are in fact at the beginning of a long road. "iPS cells as we make them today are riddled with viruses," says Daley. He says that these viruses can be mutagenic and have the potential to activate oncogenes, so at the moment iPS cells remain a research tool and not a potential therapeutic agent. But the next step for iPS cells could move them closer to therapeutic applications. "One of the next big milestones will be making these cells without the use of viruses — leaving the cells in a genetically pristine state," he says. Robert Lanza from Advanced Cell Technologies in Worcester, Massachusetts agrees and even sees routes to creating iPS cells without genetic modification. "You can potentially use fusion proteins or small molecules — there are many ways to skin the cat here."

But even if iPS cells can be created without genetic modifications, the question that researchers are asking now is, do these cells really have the same properties and potentials as embryonic stem cells? "I am very excited about iPS cells but now we need to look very carefully at the properties of these cell lines," says Martin Pera of the University of Southern California in Los Angeles. Pera says that these cells might differ in their abilities to differentiate in the same way that embryonic stem cells seem to. "If you have to make ten lines for each patient — is patient-specific therapy really realistic or will large banks of iPS cells that are tissue typed be required?"asks Pera.

Only time will tell in what directions iPS cells might be taken for basic research or for clinical applications. And while iPS-cell properties are being studied and new methods to derive these cells without genetic modifications are being created, human embryonic stem-cell research will continue. Pera says that this is the best way to proceed at the moment. "We need to move forward on both fronts." N.B.

COMPANY	PRODUCTS/ACTIVITY	LOCATION	URL
Stem-cell therapeutics development			
Aastrom Biosciences	Developing autologous stem-cell therapies for use in regenerative medicine	Ann Arbor, Michigan	www.aastrom.com
Advanced Cell Technology	Working to produce embryonic stem cells that are immunologically compatible with patients; stem-cell therapeutic development	Los Angeles, California	www.advancedcell.com
Amaxa Biosystems	Reagents for transfection of adult and embryonic stem cells	Gaithersburg, Maryland	www.amaxa.com
Cellartis	More than 30 human embryonic stem-cell lines for drug discovery, toxicity testing and regenerative medicine	Goteborg, Sweden	www.cellartis.com
ES Cell International	Stem-cell lines including clinic-grade human embryonic stem cells	Helios, Singapore	www.escellinternational.com
Geron	Developing human embryonic stem-cell-based therapy to treat spinal-cord injury	Menlo Park, California	www.geron.com
International Stem Cell Corporation	Working on deriving stem cells similar to embryonic stem cells without the need for fertilized embryo; somatic-cell nuclear-transfer technology	Oceanside, California	www.internationalstemcell.com
Novocell	Developing insulin-producing cells from embryonic stem cells as a diabetes treatment	San Diego, California	www.novocell.com
Osiris Therapeutics	Developing and commercializing cell therapies based on adult stem cells	Baltimore, Maryland	www.osiristx.com
PrimeCell Therapeutics	Working on stem-cell engineering to optimize the therapeutic potential of adult stem cells	Irvine, California	www.primegenbiotech.com
Reinnervate	Developing scaffolds for three-dimensional stem-cell culture; identifying compounds that promote differentiation of stem cells	Durham, UK	www.reinnervate.com
Stemagen	Developing methods for the derivation and propagation of human embryonic stem cells for therapeutic use	La Jolla, California	www.stemagen.com
StemCells	Investigating the use of neural stem cells for treatment of neurodegenerative diseases	Palo Alto, California	www.stemcellsinc.com
Stem Cell Innovations	Developed the stem-cell platform PluriCells for use in drug discovery application and disease modelling	Houston, Texas	www.stemcellinnovations.com
VistaGen Therapeutics	Uses embryonic stem cells for drug discovery and development	South San Francisco, California	www.vistagen.com
Stem-cell media, serum and reagent suppliers			
BD Biosciences	Feeder-free media, reagents and matrices for culturing stem cells	Franklin Lakes, New Jersey	www.bd.com
BioProcessors	Developing high-throughput miniaturized cell-culture systems	Woburn, Massachusetts	www.bioprocessors.com
Cellular Engineering Technologies	Stem-cell research, bioassay development and drug discovery; differentiation and expansion media for stem cells	Coralville, Iowa	www.celleng-tech.com
Glycosan BioSystems	Supplies HyStem hydrogels which are chemically defined and animal-free for three-dimensional cell culture of stem cells	Salt Lake City, Utah	www.glycosan.com
Invitrogen	Developing engineered reporter stem-cell lines; reagents and media for stem cells; three-dimensional matrices for stem-cell culture	Carlsbad, California	www.invitrogen.com
Lonza	Adipose-derived stem-cell lines along with media and supplements	Basel, Switzerland	www.lonza.com
Millipore	Human and rodent stem-cell lines, serum-free media, reagents, differentiation kits	Billerica, Massachusetts	www.millipore.com
Miltenyi Biotec	Products for the separation, analysis and culturing of stem cells	Gladbach, Germany	www.miltenyibiotec.com
PAA	Media, serum and reagents for stem-cell culture	Pasching, Austria	www.paa.com
ThermoFisher Scientific	Media and reagents for murine embryonic stem cells and adult stem cells; adult stem-cell lines; differentiation systems	Waltham, Massachusetts	www.thermofisher.com
R&D Systems	Kits for the differentiation of mouse embryonic stem cells to neurons or oligodendrocytes; media, antibodies and other stem-cell reagents	Minneapolis, Minnesota	www.rndsystems.com
StemCell Technologies	Media, reagents and kits for the propagation and differentiation of human and mouse embryonic stem cells and human adult stem cells	Vancouver, British Columbia	www.stemcell.com
StemCell Services	Contract provider of stem-cell services including isolation, characterization, differentiation and expansion of cells	Glasgow, UK	www.stemcell-services.com
Zen-Bio	Media for the propagation of adult stem cells and adult stem-cell lines	Research Triangle Park, North Carolina	www.zen-bio.com
Cell imaging and sorting			
Carl Zeiss	Microscopes, digital imaging of cells, image-analysis software	Jena, Germany	www.zeiss.com
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Dako North America	Automated cellular imaging, fluorescent activated cell-sorting instruments and kits	Carpinteria, California	www.dakousa.com
Hamamatsu	Imaging systems	Hamamatsu City, Japan	www.hamamatsu.com
Improvision	3D imaging software	Coventry, UK	www.improvision.com
Leica Microsystems	Light microscopes, stereomicroscopes and confocal microscopes; image-analysis software	Wetzlar, Germany	www.leica-microsystems.com
Intracellular Imaging	Digital fluorescence imaging and photometry systems	Cincinnati, Ohio	www.intracellular.com
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Micro Video Instruments	Microscopes and accessories	Avon, Massachusetts	www.mvi-inc.com
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Nanoptek	Digital tunnelling microscopes	Concord, Massachusetts	www.nanoptek.com

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Omega Optical	Optical filters and coatings for imaging; custom filters	Brattleboro, Vermont	www.omegafilters.com
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Princeton Instruments	Equipment for imaging and spectroscopy	Trenton, New Jersey	www.princetoninstruments.com
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Alexis Biochemicals	Suppliers of reagents for molecular and cell-biology research	Lausanne, Switzerland	www.alexis-corp.com
Beckman Coulter	Automated tools for genomics and proteomics research	Fullerton, California	www.beckmancoulter.com
BioFlow Technology	Developing bioreactor systems for the culturing of cells in a three-dimensional enivronment	Novi, Michigan	www.bioflowtech.com
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Merck	Chemicals, kits and reagents for molecular and cell-biology-related research	Darmstadt, Germany	www.merck.de
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Cloning Tools to Transform Results

Image courtesy of Cleaver Scientific Ltd.

The technique of cloning plays a myriad of roles in molecular biology today, with every cloning-based experiment requiring the same four steps: isolation, ligation, transfection, and selection. The variety of products available enables researchers to use comprehensive kits that support all four steps, or specific products for each element of the workflow, affording a more customized approach to molecular cloning.

Isolation

Excision of spots and bands from gels for cloning can present challenges in precision, speed, and contamination. **Cleaver Scientific** now offers standard and sterilized GelX tips, a range of disposable gel excision tips designed for use with agarose and protein gels that eliminate the need for scalpels. The GelX tip fits on a 1000 µl pipette, enabling single-handed selection, extraction, and ejection. GelX tips come in two sizes (4 or 6.5 mm x 1 mm) and are autoclavable, DNase and RNase free, and can be purchased in packs of 250 that are either bagged or racked.

To overcome the limitations imposed by conventional cloning techniques, **OriGene** offers the genome-wide TrueORF product line of ORF clones, eliminating the need for *de novo* cloning. TrueORF clones enable expression of encoded transcripts as tagged proteins, facilitating downstream applications with anti-tag antibodies. Accuracy is ensured through high fidelity polymerases, purified plasmid templates, and minimal numbers of PCR cycles. All TrueORF inserts are housed in a pCMV6-Entry vector with Myc-Flag® tags and can be shuttled by a cut-and-paste mechanism into OriGene PrecisionShuttle Destination Vectors for flexible tagging options.

Fermentas has recently introduced the FastDigest® line of restriction enzymes, which offers a single buffer system for over 130 enzymes enabling any combination of restriction enzymes to work simultaneously in a single tube. The universal FastDigest® buffer is also compatible with main downstream cloning steps, eliminating the need for buffer change between digestion, blunting, dephosphorylation and ligation. In addition, digestion times are dramatically reduced for all types of template DNA down to 5 minutes. The FastDigest® enzymes are produced in certified Class D clean room environment, ensuring a high standard of purity for even the most demanding applications.

Ligation and Transfection

Cloning projects that require the use of long DNA fragments are often fraught with complicated steps to ensure accuracy and reproducibility. **Takara's** DNA Ligation Kit LONG is a powerful tool for cloning DNA fragments over 10 kb in length for applications such as vector construction and construction of BAC libraries. The kit contains an optimized ligase/buffer system that enables ligation of long fragments without difficult techniques or special expertise. Each kit enables 50 reactions for cohesive-end or 10 reactions for blunt-end ligations.

To address cloning ligation needs, **USB** offers the Ligate-IT™ Rapid Ligation and Blunt- IT™ Repair Kits. The Ligate-IT Rapid Ligation Kit facilitates ligation of cohesive ends in 5 minutes and blunt ends in 10 minutes following incubation at room temperature. The Blunt-IT Repair Kit transforms DNA overhangs into blunt ends, using the Klenow fragment of *E. coli* DNA Polymerase I to fill 5' and digest 3' overhangs. The resulting DNA can be quickly ligated utilizing the Ligate-IT Rapid Ligation Kit. USB also offers SuperSAP™, shrimp alkaline phosphatase, to reduce background by dephosphorylating blunt-ended vectors in only 5 minutes.

The Fast-Link™ DNA Ligation Kit from **EPICENTRE** uses a ligase that was cloned and formulated for rapid, high-efficiency

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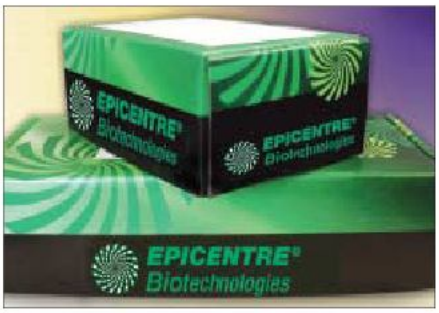

Figure 1. EPICENTRE offers the Fast-Link™ DNA Ligation Kit for rapid, high-efficiency DNA ligation with both routine and high-throughput cloning applications.

Figure 2. The Enviro-Genie® from Scientific Industries uses Peltier technology for precise temperature control and is compatible with an assortment of culture vessels.

DNA ligation, offering compatibility with both routine and high-throughput cloning applications. Cohesive-end ligations can be performed in 5 minutes at room temperature, while blunt-end ligations take just 15 minutes. The desalt step has been eliminated from Fast-Link ligation reactions prior to transformation of electrocompetent or chemically competent cells.

Lipofectamine™ LTX Reagent from **Invitrogen Corporation** offers a solution for gene expression studies in primary and hard-to-transfect cells. Lipofectamine LTX Reagent offers a streamlined protocol that eliminates the need to remove transfection complexes or adjust medium volumes following transfection reactions.

Cloning Kits

The Director™ Universal Cloning System from **Sigma-Aldrich Co.** enables directional cloning of PCR products into vectors cleaved with 5' overhang-producing restriction endonucleases. Following PCR, cohesive 5' termini of the amplicon are generated by Exonuclease III digestion rather than traditional restriction enzymes, offering protection from over-digestion. In addition, the nucleotide mix in the kit is formulated for specific amplicon termination at a statistically determined array of 3' dA/G α S sites and PCR primers are designed to ensure that the 5' termini complement the 5' overhangs of the predigested plasmid. Using this kit, the three-step procedure of PCR, Exonuclease III digestion, and rapid ligation/transformation can be completed in one day, with greater than 80% average cloning efficiency.

The **QIAGEN** PCR Cloning plus Kit enables fast and highly efficient cloning of PCR products generated using *Taq* and other non-proofreading DNA polymerases. The pDrive Cloning Vector provides efficient cloning of PCR products through UA hybridization in less time than is required for TA-based cloning vectors. A pre-optimized, ready-to-use Ligation Master Mix, QIAGEN EZ Competent Cells, and SOC medium are also supplied in each kit for added convenience.

Clontech Laboratories' In-Fusion™ PCR Cloning Kit enables PCR fragments to be inserted at any cleavage position within any plasmid, by fusing linear DNA fragments with a short region of overlapping homology at their ends. Using the kit, there is no need for dephosphorylation, ligation, or blunt-end polishing. Kits contain all required reagents, including linearized vector control insert. The system is also available in a unique lyophilized format that's ideal for high-throughput applications.

Cloning Workstations and Incubation Systems

Many automated air-filled liquid handling systems do not address potential contamination from aerosols or splashing during sample transfers. Tecan's Freedom EVO® robotic workstation with Flow-Thru Technology addresses internal cleanliness by washing the pipetting channel as needed in a two-step process that cleans the exterior and interior of fixed washable tips or disposable tip cones. Powered by the intuitive software, Freedom EVOware®, each system can automate genomic, proteomic, drug discovery, and other life science applications. Tecan offers the Freedom EVO platform in four different base sizes (75, 100, 150 and 200 cm) with a variety of options.

Sartorius Stedim Biotech has engineered the CERTOMAT® range of heavy-duty incubation shakers specifically geared for long-running, heavy load microbiological growth requirements. In any laboratory regularly cloning for the purposes of recombinant protein or DNA expression, these incubation shakers are a valuable tool for expanding cultures reproducibly. A comprehensive variety of drive types, capacities, user interfaces and adapters are available.

The **Scientific Industries** Enviro-Genie® is a bench-top environmental chamber that is temperature controlled using Peltier technology for precise temperature control from 4°C to 75°C and is compatible with an assortment of culture vessels. In addition, the Enviro-Genie enables sample manipulation through stirring, rocking, rotating, and/or gyrating motions. The chamber is programmable to 99 hours and has visible and audible alarms for time and temperature monitoring.

"As the number of gene-based disease linkages continues to grow, researchers demand wide access to full-length clones, delivered ASAP and ready-to-use, for easily accessible HTP screening and simplified protein tagging."

Karl Kovacs, Ph.D.
VP, Customer Services
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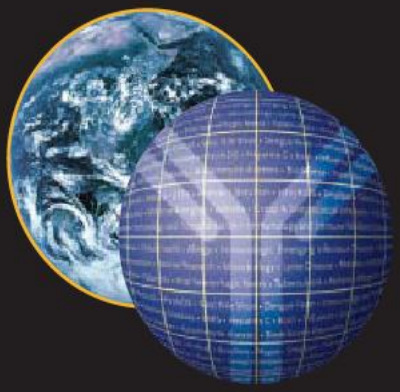

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An outstanding postdoctoral record of research accomplishment and M.D., M.D. /Ph.D. or equivalent degree is required for this position; board eligibility/board certification is also required. The incumbent will be expected to be qualified for credentialing by the NIH Clinical Center.

Candidates will be assigned independent resources to include clinical and/or laboratory support personnel, equipment, space, and an allocated annual budget for services, supplies, and salaries to ensure success. This is a tenure-track appointment under Title 42. Salary is dependent on experience and qualifications.

Interested candidates may contact Dr. Karyl Barron, Deputy Director, DIR, NIAID at 301/402-2208 or email (kbarron@nih.gov) for additional information about the position.

To apply for the position, send your curriculum vitae, bibliography, and an outline of your proposed research program (no more than two pages), by **March 14, 2008** via email to Ms. Wanda Jackson at jacksonwa@niaid.nih.gov. In addition, three letters of recommendation must be sent to Chair, NIAID DIR Clinical Tenure Track Search Committee, c/o Ms. Wanda Jackson at jacksonwa@niaid.nih.gov or 10 Center Drive MSC 1356, Building 10, Rm. 4A-26, Bethesda, Maryland 20892-1356. E-mail is preferred. Please note search #018 when sending materials.

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Graduate students at several US universities and colleges are trying to form unions, with mixed success. According to the Coalition of Graduate Employee Unions, students already have collective-bargaining rights at more than 20 US schools (see *Nature* **428,** 965; 2004). But is unionizing a good idea? Certainly, teaching and research assistants deserve a say in their pay, work benefits and conditions. But should students engage in collective bargaining and risk potentially unproductive clashes with administrators?

Students at the University of Maryland in College Park are the latest to campaign for worker rights. A state legislator named Jamie Raskin has introduced a bill that would allow graduate students and adjunct professors to unionize at state-funded universities. He is quoted in *The Washington Post* as saying that graduate students are treated like "migrant workers". Union proponents say that students who work for professors or teach undergraduates should have a seat at the bargaining table and a say in their working conditions.

A spokesperson from the university counters that students are just that, students, not employees. Perhaps this is an overly rigid stance, meant to save the university money and time at the negotiating table. But unions are not necessarily a positive step for students. The employer–employee relationship differs from that of the teacher–student. The former can be adversarial, and counter to the aims of a graduate student. US students have their hands full just being students, with degrees that can take 6–8 years to complete. They might not have the time or the patience to deal with lawyers and salary disputes that could, after a long struggle, net only minor gains.

Yet in the times of tight budgets and large labs, graduate students have the right to make sure they're not merely cheap labour helping their professor to get tenure, publications or increasingly rare grant money. And graduate student unions can be good for the university — prospective students might see unionization as a positive attribute. But, considering the pitfalls, students should be careful not to focus too much on being employees — the primary aim should be a degree, not a union card.

Gene Russo, acting editor of *Naturejobs*

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MOVERS

Alex Dehgan, senior scientist and policy adviser, US Department of State, Washington DC

2006–07: Afghanistan country director, Wildlife Conservation Society, Kabul
2006: Member, policy-planning staff, Office of the US Secretary of State, Department of State, Washington DC
2005–06: Senior science fellow, World Conservation Union, USA Multilateral Office, Washington DC

As a young man, Alex Dehgan's diverse interests spanned conservation biology, environmental science, law and international trade. He ultimately pursued each, and, inadvertently, prepared for a career in foreign policy. His circuitous route has taken him from Madagascar to Iraq as he engaged people across disciplines as well as borders.

Dehgan graduated from Duke University in Durham, North Carolina, with a double major in zoology and political science. "Either way, I figured I was dealing with animal behaviour," he says. A trip to Madagascar for an undergraduate course in primate conservation influenced his future career path.

After earning a law degree to "better understand the social fabric that holds society together", Dehgan decided to return to conservation. He began his PhD at the University of Chicago, and later returned to Madagascar to study lemur populations in fragmented forestlands for his dissertation.

Despite his love of fieldwork, Dehgan decided to enter the policy realm to help conserve tropical forests. An American Association for the Advancement of Science policy fellowship placed him at the US Department of State. Although Dehgan had difficulty finding a niche, Norman Neureiter, the first scientific adviser to the state department, was so impressed with his background that he persuaded Dehgan to continue. "Alex's diverse interests and expertise were exactly what US foreign policy needs," says Neureiter, now the director of the Center for Science, Technology and Security Policy in Washington DC.

Dehgan was ultimately sent to Iraq to retrain weapons scientists in fields such as ecology and conservation biology. He also led efforts to establish the Iraq International Center for Science and Industry and reconstruct Iraq's Natural History Museum. And he supplied Iraqi scientists with electronic access to hundreds of scientific journals. "Science is one area that cuts across culture to engage people — why not integrate it into diplomacy and development?" he says. He later went to Afghanistan to conduct the first wildlife surveys in 30 years, collaborating with a team of native, yet novice, naturalists.

In his latest role as a senior policy adviser to the US Secretary of State's newest science adviser, Nina Federoff, Dehgan hopes to use science to buoy developing countries' economies and emphasize the relevance of climate change in foreign policy and nation-building strategies. ■
Virginia Gewin

NETWORKS & SUPPORT

Lab links with southern states

The US Department of Energy's Oak Ridge National Laboratory (ORNL) in Tennessee last week signed a 'mentor–protégé agreement' with Jackson State University (JSU) in Mississippi. As part of the three-year commitment, ORNL will serve as mentor to the entire school, and collaborations will be forged across research sectors common to the pair. ORNL will be able to award non-competitive subcontracts, or form collaborations to submit funding proposals to other agencies.

ORNL, which made a similar agreement with Morehouse College in Georgia last year, is the first national science laboratory to forge collaborative agreements with historically black institutes.

"If we can help our subcontractors find synergies with our researchers, better understand our contracting mechanisms and learn to draft solid proposals, they'll be better partners," says Will Minter, director of ORNL's small-business programmes.

Although the JSU agreement is new, the two institutions already have collaborations in computational chemistry, homeland security, biological sciences and nanomaterials. JSU computational chemist Jerzy Leszczynski has worked with ORNL researchers on carbon nanostructures. His project is one of several that should grow with larger funds from ORNL in the future. "We're not expecting Oak Ridge to give us a $10-million cheque. We want the opportunity to grow our strong programmes," says Rita Presley, JSU's associate vice-president for research and sponsored programmes. Presley hopes a strong relationship with ORNL will help JSU secure more mainstream funding, not necessarily money set aside for historically black institutes.

Morehouse College administrators say that access to ORNL equipment, such as its supercomputing capabilities, will soon help them to do research with a national scope. Noting that ORNL's biofuels research is looking at cellulose conversion, James Brown, director of Morehouse's Office of Research Careers, contacted Duane Jackson, who studies termites — efficient cellulose converters. Jackson is now working with a Morehouse chemist and botanist to identify useful termite gut enzymes, which will feed into ORNL's biofuels work.

ORNL continues to seek partners eager to forge research partnerships. Minter says that other national labs seem interested in tracking the progress of ORNL's collaborations. "This programme is an experiment, but that's what labs do," he says. ■
Virginia Gewin

POSTDOC JOURNAL

Serial postdoc

It's a bad habit to get into. Postdoc positions are liberally scattered over the past 10 years of my CV, and it's got to stop. Well, something has to change, because my funding runs out in 12 months. So here's the deal: I find a permanent position by the end of the year or I move out of academia... Gulp.

It's hard to believe that it's been 10 years since I finished my PhD in cosmology. It's been 10 years since I made a galactic research leap and followed my growing interest in biology. And I've conducted 10 years of research in mathematical ecology and evolutionary biology, which has taken me to four countries and facilitated collaborations with a diverse array of biologists. At the start it was hell. But the collaborations gave me a research foothold. Now, looking back on seabirds, cows, sheep, frogs, DNA and equations, the range of problems and experiences has been a joy. Theoretical ecology brings a lot of job diversity.

So here I am, a well-travelled senior postdoc at the University of Lausanne. Lectureship applications have started, job searching is well under way, as is considering where my partner and I could live. Have I been a postdoc too long? Have I collaborated too much? And what if this career doesn't work? In the year to come I'll have to explore the possibilities and contact friends who have made the move out of academia. Kick the postdoc habit. ■

Jon Yearsley is a senior postdoc in evolutionary genetics at the University of Lausanne in Switzerland.

Tenure-Track/Tenured Position
Clinical Translational Research

The Division of Intramural Research of the National Heart, Lung and Blood Institute seeks an individual to direct an independent clinical or translational research program in the heart, lung or blood diseases. The successful candidate would develop a program to complement and integrate with existing research in Bethesda. An M.D. or M.D./ Ph.D. degree and record of research accomplishments as evidenced by publications in major peer-reviewed journals are required. This position comes with generous start up funding, as well as stable intramural personnel and budget support. There is access to advanced core facilities, including large research hospital at the NIH Clinical Research Center; a pharmacy development service; a transgenic and knockout mouse facility; confocal and electron microscopy cores; a multi-modality non-invasive mouse imaging facility; siRNA resources; an advanced fluorescent activated cell sorting facility; and state of the art genomics, microarray and proteomics.

A competitive salary commensurate with experience and qualifications is offered. **Appointees may be US citizens, resident aliens or non-resident aliens with or eligible for a valid employment visa**. Applications must be received by **February 15, 2008**. Please submit a curriculum vitae and brief statement of research interests along with three letters of reference to:

Neal S. Young, MD, Chief, Hematology Branch, NHLBI, NIH, c/o Ms. Sheree Hawkins, Building 10, Room 7N220, 10 Center Drive, Bethesda, MD 20892.

You may apply using one of the following methods:

1. Mail application package to address listed above
2. Email your application to **hawkinss@mail.nih.gov**
3. Apply on-line at **http://www.training.nih.gov**
    a. click on NIH Science Jobs
    b. click on Current NIH Tenure-Track and Tenured Openings
    c. click on Tenure Track-Tenured Position Clinical Translational Research
    d. click on Apply

Program Officer

The National Institute of Mental Health (NIMH) seeks candidates for a Program Officer position to develop and manage a grants portfolio in human genetics research on mental disorders. With the completion of the Human Genome Project and accompanying advances in the development of new genetic tools and technologies, there are unprecedented opportunities to discover genes that produce vulnerability to mental disorders. This research program at NIMH is intended to accelerate the discovery of such genes and characterize the genetic basis of disorders directly relevant to NIMH's mission, through the application of cutting edge genomic-based approaches and methods. The research supported by this program will accelerate the development of pharmacogenomics, diagnostics, therapeutics and effective prevention strategies for mental disorders. Responsibilities will include developing new research initiatives, administering and managing an extramural portfolio of research awards, and interacting with researchers and related programs at NIMH, NIH, and other funding agencies (both public and private, in the U.S. and abroad). Candidates must be a U.S. citizen and have an M.D., Ph.D., or equivalent-level degree and considerable research experience in human genetics. Preference will be given to candidates with experience in research management. Experience in genomics-based genotyping, target validation and drug discovery is highly desirable. The ability to work both independently and collaboratively is required. Strong communication, writing and organizational skills are also required. The position will be filled on a permanent basis. Salary will be commensurate with experience. **Send CV, bibliography and the names of 4 references by email to Dr. Thomas Lehner at tlehner@mail.nih.gov (Tel: 301-443-9869; Fax: 301-451-5615)**. With nationwide responsibility for improving the health and well being of all Americans, the Department of Health & Human Services oversees the biomedical research programs of the National Institutes of Health (**http://www.os.dhhs.gov**).

Tenure-Track Position

The Center for Population Studies in the National Heart, Lung, and Blood Institute (NHLBI) at the National Institutes of Health is inviting applications for a tenure track scientist to develop an independent research program in metabolic risk factors for cardiovascular disease. Applicants should possess an M.D, and M.P.H, and training in the field of internal medicine and endocrinology, an outstanding record of achievements in research, and excellent publication history, evidence of ongoing mentoring of junior investigators, and provide evidence of contributing to the development of their discipline. We seek a talented individual who has a clear research vision in the application and development of state-of-the-art methods to study metabolic risk factors for cardiovascular disease.

The intramural program of NHLBI is dedicated to maintaining an excellent research environment with 5 Basic Research Centers and 3 Clinical/Translational Branches comprising 64 principal investigators working on a wide spectrum of research topics. The new position will add to the current Center for Population Studies research strengths of the institute that include the Framingham Heart Study and the Computed Tomography CT Sub-study, the Jackson Heart Study, the Hispanic Study, the SHARe project, and the SABRe project. The position comes with generous start up support and ready access to state of the art core facilities, including a core laboratory, a DNA laboratory, a core computed tomography laboratory, and a statistical core. In addition to leading a vigorous and independent research program, the successful candidate will take an active role in continuing to build the metabolic program in the Institute.

The successful candidate will be offered a competitive salary commensurate with experience and qualifications. This position is located in Framingham, Massachusetts. Appointees may be US citizens, resident aliens, or non-resident aliens with or eligible to obtain a valid employment visa. Applications must be received by March 1, 2008. Please submit a curriculum vitae and brief statement research interests along with three letters of reference to:

Daniel Levy, MD, Director, Framingham Heart Study, Director, Center for Population Studies, c/o Sandra Stoddard, The Framingham Heart Study, National Institutes of Health, 73 Mt Wayte Ave Suite #2, Framingham MA 01702.

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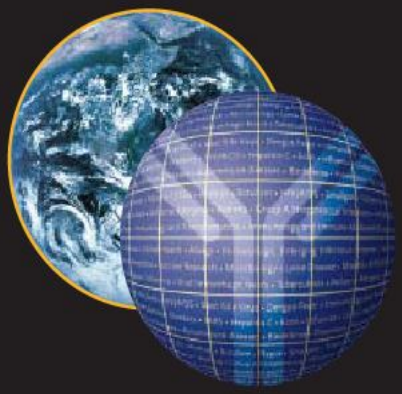

NIAID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Help Us Help Millions

Clinical Tenure-Track Position

The National Institute of Allergy & Infectious Diseases (NIAID), Division of Intramural Research (DIR), is seeking an outstanding tenure-track investigator to develop a clinical research program to better understand, treat, and ultimately prevent infectious, immunologic, and/or allergic diseases. The scope of the NIAID research portfolio has expanded considerably in recent years in response to new challenges such as bioterrorism; emerging and reemerging infectious diseases, including acquired immunodeficiency syndrome (AIDS), influenza, severe acute respiratory syndrome (SARS), West Nile virus, malaria, and tuberculosis; immunologic diseases and the increase in asthma prevalence among children in this country.

The successful candidate will implement and direct an independent clinical research program with research emphasis on clinical research but may include translational or basic research. The incumbent will have the opportunity to choose the Laboratory in which he/she would like to be affiliated. It is expected that clinical protocols developed will complement the research goals of the Laboratory selected. In addition, the candidate will be paired with a Senior Investigator who will serve as a clinical mentor.

An outstanding postdoctoral record of research accomplishment and M.D., M.D. /Ph.D. or equivalent degree is required for this position; board eligibility/board certification is also required. The incumbent will be expected to be qualified for credentialing by the NIH Clinical Center.

Candidates will be assigned independent resources to include clinical and/or laboratory support personnel, equipment, space, and an allocated annual budget for services, supplies, and salaries to ensure success. This is a tenure-track appointment under Title 42. Salary is dependent on experience and qualifications.

Interested candidates may contact Dr. Karyl Barron, Deputy Director, DIR, NIAID at 301/402-2208 or email (kbarron@nih.gov) for additional information about the position.

To apply for the position, send your curriculum vitae, bibliography, and an outline of your proposed research program (no more than two pages), by **March 14, 2008** via email to Ms. Wanda Jackson at jacksonwa@niaid.nih.gov. In addition, three letters of recommendation must be sent to Chair, NIAID DIR Clinical Tenure Track Search Committee, c/o Ms. Wanda Jackson at jacksonwa@niaid.nih.gov or 10 Center Drive MSC 1356, Building 10, Rm. 4A-26, Bethesda, Maryland 20892-1356. E-mail is preferred. Please note search #018 when sending materials.

Further information regarding the DIR laboratories is available at: http://www3.niaid.nih.gov/about/organization/dir/default.htm and information on working at NIAID is available on our website at: http://healthresearch.niaid.nih.gov/tdir

Department of Health and Human Services
National Institutes of Health
National Institute of Allergy and Infectious Diseases

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NIAID

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HELP US HELP MILLIONS

Research Fellow/Senior Research Fellow Positions:

***Staphylococcus* pathogenesis and/or innate immunity**

Dr. Michael Otto or Dr. Frank DeLeo

The **National Institute of Allergy and Infectious Diseases** (NIAID), a major component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is recruiting **two Research Fellows/Senior Research Fellows** in the Laboratory of Human Bacterial Pathogenesis (LHBP). Both positions may be filled as either a Research Fellow or a Senior Research Fellow, dependent upon applicants' qualifications.

The purpose of a Research Fellowship is to provide junior-level scientists with doctoral degrees experience in biomedical research while they provide a service relevant to the NIH's program needs. The Research Fellow will spend the entire fellowship in laboratory research, while supporting the performance of NIH intramural research. To be eligible for the Research Fellowship, a candidate must have demonstrated outstanding scholastic achievement and the ability to conduct successfully, with minimal supervision, a pre-established program in laboratory research.

The research activity primarily involves the investigation of *Staphylococcus* molecular pathogenesis and interaction of *Staphylococcus* with the human host, using in vitro and in vivo molecular biology, biochemistry and immunology approaches.

Applicants for these Research Fellow positions should have a Ph.D. and at least 3-5 years of post-doctoral work experience, preferably in *Staphylococcus* or Gram-positive bacterial pathogenesis research, or experience in the area of innate immunity or host-pathogen interactions. Scientists with considerable experience beyond postdoctoral training may be designated as a Senior Research Fellow. The Senior Research Fellow position is equivalent to a non-tenure track position in a university setting, e.g. Research Associate, Research Assistant Professor.

These positions will be located on the NIH main campus in Bethesda, Maryland (Dr. Otto) or at Rocky Mountain Laboratories, Hamilton, Montana (Dr. DeLeo), and subject to the 5-year/8-year limitation at the NIH. However, future conversion of a Research Fellow/Senior Research Fellow into a Staff Scientist position is possible. These positions are available to foreign nationals, but thorough proficiency in the English language in reading and writing is required. Entry level salaries are in the range of USD 42,897 – 79,127 for Research Fellow and USD 74,503 – 110,238 for Senior Research Fellow, depending on qualifications. In exceptional cases, higher tier entry levels are negotiable.

Applicants should submit their curriculum vitae including bibliography, letter of research interests, and names and addresses of three references to: **Penny-Gaddy Rhodes**, Rocky Mountain Laboratories, Laboratory of Human Bacterial Pathogenesis, 903 South Fourth Street, Hamilton, Montana, 59840 or **Michael Otto, Ph.D.**, Chief, Pathogen Molecular Genetics Section, LHBP, NIAID (motto@niaid.nih.gov) or **Frank R. DeLeo, Ph.D.**, Chief, Pathogen-Host Cell Biology Section, LHBP, NIAID (fdeleo@niaid.nih.gov).

For more information about NIAID and to view additional job opportunities, please visit: **http://healthresearch.niaid.nih.gov/lhbp**

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National Institutes of Health
National Institute of Allergy and Infectious Diseases
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TRINITY COLLEGE DUBLIN

The University of Dublin

www.tcd.ie/vacancies

Trinity College is recognised internationally as Ireland's premier university and is the only Irish university to rank in the top 100 world universities (53rd) and amongst the top 50 European universities (13th). We are recruiting world class leaders in research and education to advance our research strengths, develop our fourth level graduate education and build on our excellence in third level undergraduate education. Our strategic priorities of research and education are also aligned with contributing to the national goal of fostering Ireland's cultural and economic vibrancy.

School of Chemistry

Lectureship in Inorganic Chemistry (permanent)

The School of Chemistry has an excellent track record in attracting funding from competitive research programmes such as those operated by the Irish Higher Education Authority, the E.U. and Science Foundation Ireland. It is a key partner in several College-based initiatives in areas including molecular synthetic chemistry, nanomaterials and computational chemistry.

Candidates for this post should have a strong background in inorganic research, holding a Ph.D. in a relevant area of Chemistry and have several years' postdoctoral research experience. They will undertake independent research in synthetic Inorganic/Bioinorganic or Materials Chemistry and contribute to the undergraduate and postgraduate teaching programmes within the School, including lecture and practical courses at all levels in Inorganic Chemistry. Opportunities exist for individuals to undertake national, international and interdepartmental collaborations and the School is particularly well-equipped to support modern inorganic research.

Requests for further information relating to the position can be addressed to Professor S. Draper, Head of Inorganic and Synthetic Materials Chemistry, smdraper@tcd.ie or to the Head of School, Professor D. H. Grayson, headchem@tcd.ie. Alternatively requests can be submitted to adchem@tcd.ie

Appointment will be made on the Lecturer salary scale: €36,433 - €73,527 / €75,976 - €83,512. Commencing salary will be at a maximum of the 8th point: €51,407 per annum. The preferred start date is 1st October, 2008. Closing date for applications is no later than 12 noon on Monday, 31st March, 2008.

Further information and full application details can be found at www.tcd.ie/vacancies

Trinity College Dublin is an equal opportunities employer.

W125061R

Scientific Copy Editors

Cambridge, UK

The Company of Biologists (www.biologists.com) is a not-for-profit organisation, publishing several distinguished scientific journals: *The Journal of Experimental Biology*, *Journal of Cell Science*, *Development* and, coming soon, *Disease Models & Mechanisms*. The organisation has an active programme of charitable giving for the further advancement of biological research, including travelling fellowships for junior scientists and contributions to academic societies and conferences.

We currently have two vacancies available:

The Journal of Experimental Biology

The leading journal in comparative animal physiology, *The Journal of Experimental Biology* publishes papers on the form and function of living organisms at all levels of biological organisation, from the molecular and subcellular to the integrated whole animal. We are looking for an experienced editor to copy edit articles to a high standard, to compile and paginate articles within an issue, to oversee the journal production process and to liaise with authors, academic editors and production staff to ensure that articles are published in print and online in a timely and professional manner.

DMM Disease Models & Mechanisms

Our new journal, *Disease Models & Mechanisms*, covers a rapidly developing field – the use of model organisms to understand the mechanisms underlying human disease and to develop novel diagnostics and therapeutics. We are offering an exciting opportunity to join the editorial team launching this new journal to the scientific community. The position involves copy editing articles to a high standard and to strict deadlines, compiling articles within an issue and liaising with authors, our US-based editors and in-house production staff. The successful candidate will also be a proven team player and show a general enthusiasm to be involved in various aspects of a launch project.

Candidates for either position should have a degree or PhD in a relevant scientific field, and previous copy editing experience is essential. Additional requirements include excellent literacy skills, high attention to detail, a diplomatic communication style, good interpersonal and IT skills, a flexible approach and the ability to work to tight deadlines.

Both posts are full-time (although part-time working may be considered) and are available from early April. They represent an excellent opportunity within science publishing and offer an attractive salary and benefits.

Applicants should send a CV along with a covering letter that clearly states which post they are applying for, their current position and salary, relevant experience and why they are enthusiastic about the post.

The Company of Biologists

Applications should be sent by email no later than 28 February to miriam@thecob.demon.co.uk
Informal queries to Miriam on 01223 426164

W125063R

Call for Research Proposals

"Ajinomoto Amino Acid Research Program"

Amino acids serve multiple roles in the biological system. Ajinomoto Co., Inc., which is the leading company on the production and uses of amino acids worldwide, is interested in supporting innovative research focusing on the biological aspects of amino acids, such as nutritional, physiological and/or pharmacological functions and properties.

Research proposals are invited for the following support categories:

- **Exploratory Research Grants :** Maximum of $100,000 per year, up to 2 years
- **Young Investigator Research Grants :** Maximum of $100,000 per year, up to 2 years

  Investigators who are within 5 years as independent investigators by the deadline of full applications are eligible for young investigator category.

Applicants must submit a pre-application to be received no later than 31st March 2008; full applications must be received no later than 1st August 2008.

For more details and instructions:
http://www.3arp.ajinomoto.com
3arp@ajinomoto.com

AJINOMOTO®

Ajinomoto Co., Inc. is pleased to announce the recipients of the 3ARP grant 2007.

Ajinomoto Co., Inc. appreciate the interest and participation of a large number of scientists. We received over 160 high-quality proposals from around the world for this program in 2007. After extensive review and deliberation, the following 8 proposals were selected for funding.

John Ngai (University of California, Berkley)
Identification of Amino Acid Ligands for Olfactory and Taste Receptors

Gerard Karsenty (Columbia University)
Amino Acids as Regulators of Bone Mass

Jochen Reiser (Massachusetts General Hospital / Harvard Medical School, *Young Investigator* **)**
Amino Acid Metabolism in Kidney Podocytes as Target for Proteinuric Kidney Disease

Thomas E. Finger (University of Colorado)
Amino Acid Ingestion and Brain Activation in "Tasteless" Mice

Douglas R. Cavener (Pennsylvania State University)
Amino Acid Sensor GCN2 Regulation of Fat Metabolism

Sue C. Kinnamon (Colorado State University)
Glutamate: A Tastant and a Neurotransmitter in Taste Buds

Naomi K. Fukagawa (University of Vermont, College of Medicine)
Synergistic Effects of the Branched Chain Amino Acids (BCAA) and Carnosine (CAR) Precursors on Exercise-induced Muscle Damage and Performance in Humans

Janet Brunton (Memorial University of Newfoundland, *Young Investigator* **)**
Dipeptides as Trophic Factors in the Gut

JP123396R

MANCHESTER 1824

The University of Manchester

Manchester Interdisciplinary Biocentre

Postdoctoral Research Associate – Systems Analysis of Translation- Computational

£26,666 - £29,139 p.a. **Ref: EPS/80016**

A BBSRC-funded Postdoctoral position is available in the Manchester Interdisciplinary Biocentre to work with Prof. John McCarthy and Prof. Hans Westerhoff. The position involves experimental rate control analysis of the eukaryotic translation initiation pathway, both in vivo and in vitro.

We seek an enthusiastic and highly capable researcher with appropriate experience in computational data handling ideally combined with experimental lab experience in molecular biology. You should have an interest in systems biology approaches, and have a track record and keen interest in computational analysis and/or model building, ideally in relation to gene expression systems. However, candidates with relevant experience in other relevant computational skills, as well as a strong commitment to working in quantitative bioscience are also welcome to apply. Additionally, some experience in experimental work on eukaryotic gene expression would be helpful.

The position will be for two years. Funding is available to support a candidate with three years' postdoctoral experience.

The work will be carried out in the newly opened Manchester Interdisciplinary Biocentre (www.mib.ac.uk) which, with support from the Wellcome Trust and the Wolfson Foundation has been constructed to accommodate a range of research at the interface between physical sciences and biology.

This project will be supported by a full-time technician.

Preliminary enquiries concerning any aspects of the project may be made to Prof John McCarthy on email: john.mccarthy@manchester.ac.uk Schools of Chemical Engineering and Analytical Sciences and Life Sciences or to Prof Hans Westerhoff on email: hans.westerhoff@manchester.ac.uk School of Chemical Engineering and Analytical Sciences.

Application forms and further particulars are available from our website or by contacting +44 (0) 0161 275 8837 or email: eps-hr@manchester.ac.uk quoting the reference number.

Closing date: 29 February 2008.

Faculty of Life Sciences

Postdoctoral Research Associate

£26,666 - £29,139 p.a. **Ref: LS/020/08**

You will work on a BBSRC-funded project that will apply methods of genetics, biophysics, biochemistry and structural biology to the study of an enleanstic involved in mRNA metabolism and its regulation. The project will examine interdependent structures function relationships, the roles of these proteins in error driven sequences, and their sub-celluar.

The above project will be based in the McCarthy group, housed in the Manchester Interdisciplinary Biocentre (www.mib.ac.uk) which, with support from the Wellcome Trust and the Wolfson Foundation, has been constructed to accommodate a range of research at the interface between the physical sciences and biology.

You should hold a PhD or equivalent and have some experience of biochemical work on a eukaryotic system.

This position is available until 31 December 2009.

Informal enquiries should be made to: Professor John McCarthy on email: john.mccarthy@manchester.ac.uk or +44 (0) 161 306 8916.

Application forms and further particulars are available from our website or by contacting +44 (0) 161 275 8836 or Lifesciences-hr@manchester.ac.uk quoting the reference number.

Closing date: 29 February 2008.

Cardiovasclar Research Group

Research Associate in Cellular Cardiology

£26,666 - £27,466 p.a. **Ref: MHS/049/08**

You will 'map' the functional and molecular properties of the sinoatrial and atrioventricular nodes of the heart. Depending on your expertise you will use a range of techniques including electrophysiology (optical mapping and possibly single cell patch clamp), biochemistry (Western blot and immunohistochemistry) and molecular biology (quantitative real-time and in situ hybridisation).

You will be highly motivated, have a keen interest in cellular cardiology, have a relevant Ph.D., have good communication skills, be able to work well both in a group and independently, and have experience in one or more of the following: electrophysiology, biochemistry and molecular biology.

The post is available for 16 months in the first instance

Informal enquiries can be made to Professor M R Boyett on +44 (0) 161 275 1192 or mark.boyett@manchester.ac.uk and Dr H Dobrzynski on +44 (0) 161 275 1182 or halina.dobrzynski@manchester.ac.uk

Application forms and further particulars are available from our website or by contacting +44 (0) 161 275 1197 or julie.heydon@manchester.ac.uk quoting the reference number.

Closing date: 29 February 2008.

The University will actively foster a culture of inclusion and diversity and will seek to achieve true equality of opportunity for all members of its community.

www.manchester.ac.uk/jobs

U125212R

U125066RL

Exeter College, Oxford

£23,692 - £30,913 p.a.

Monsanto Research Fellowship

Exeter College proposes to elect to a Fellowship in Molecular or Cellular Biology, Biochemistry or Physiology from 1 Oct 2008. The Fellowship is for a maximum of five years. You should have significant postdoctoral experience, be engaged in independent research and will be required to undertake research in a relevant Oxford University department.

Contact

Tel: (01865) 279660
Email: academic.administrator@exeter.ox.ac.uk
www.exeter.ox.ac.uk/news/vacancies

U124912RL

The Sanger Institute

Research Administrator

Research Administrator to plan ES mutagenesis projects, coordinate ES cell culture group to produce mutant ES cells. PhD in Biology, postdoctoral training, experience of molecular biology & ES cell technologies, culture of mammalian cells, gene targeting homologous recombination in mouse embryonic stem cells, bioinformatics & genomie data.

Contact

Email: recruit@sanger.ac.uk
http://www.sanger.ac.uk

U124898RL

The Sanger Institute

Targeting knockout (KO) vectors

Computer Biologist

Computer Biologist required to design targeting knockout (KO) vectors for mouse KOMP mutagenesis programs using gene structures in Ensembl & Vega. Bachelor/Masters degree in bioscience or experience in genomics/genetics field. Experience using annotation/data analysis tools/genomic browsers and understanding of mouse KO technology desirable.

Contact

recruit@sanger.ac.uk
www.sanger.ac.uk

An Exciting Opportunity in Research Strategy

science foundation ireland
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Director, Lifesciences

Science Foundation Ireland is recruiting for the position of Director, Lifesciences. The Director should be a thought-leader of international renown in the lifesciences research field. He/She is expected to have the appropriate scientific insights in order to develop and implement a strategic vision to realise SFI's mission and goals for expanding high-quality, internationally-competent research in Ireland.

Candidates must have:

- Ph.D relevant to the responsibilities of leading the Lifesciences Directorate.
- Distinguished career in international scientific research.
- Record of highly competent organisation leadership and vision.
- Demonstrated successful experience in research, grants administration and other relevant management experience and capabilities.

A full detailed job description is available on the SFI website at www.sfi.ie

If you wish to be considered for this position please email your CV, quoting the job title "Director Lifesciences" to patricia.ryder@sfi.ie or post it to:
Science Foundation Ireland
Wilton Park House
Wilton Place
Dublin 2

The closing date for receipt of applications is Friday, February 29th 2008.

SFI is an equal opportunities employer.

Research for Ireland's Future

NDP
National Development Plan 2007 - 2013

W125182R

www.sfi.ie

University of Oxford

Wellcome Trust Centre for Human Genetics

Posts in Statistical Genetics and Informatics

An exciting opportunity has arisen for 5 scientists to work at the Wellcome Trust Centre for Human Genetics in the research groups of Professor Mark McCarthy, Dr Eleftheria Zeggini and Dr Andrew Morris.

Post one (Ref: H7-08-002-MM)

£33,779 - £40,335 p.a.

We are looking for an experienced Statistical Geneticist to work with Professors McCarthy and Donnelly (Oxford) and Peltonen (Wellcome Trust Sanger Institute) within the EU-funded ENGAGE consortium (www.euengage.org). ENGAGE brings together many of the European groups most active in large-scale genetic and genomic analysis and will exploit the power of data combination (~100,000 genome wide association scans) and large-scale replication (~500,000 DNA samples) to identify and characterise variants impacting on common disease, as well as to address key methodological issues posed by such studies. You will be playing a leading role in the development, management and implementation of this high-profile international project and will therefore be expected to have a track record of achievement in the analysis of large-scale genetic data sets and experience of participating in large international collaborations.

Post two (Ref: H5-08-009-EZ)

£26,666 - £32,796 p.a.

We are seeking to appoint a Research Associate in Statistical Genetics to work with Dr. Eleftheria Zeggini. You will be appointed as part of the arc-funded arcOGEN consortium and will contribute to nation-wide efforts to map osteoarthritis susceptibility genes through a large-scale (~8,000 cases) genome-wide association scan. You will have overall responsibility for the statistical analysis of this high-profile study, including liaison with and reporting to the full range of collaborative partners. We are looking to appoint someone with a strong record of academic achievement including a relevant postgraduate degree. Experience in the management and analysis of large genetic datasets is essential.

Post three (Ref: H5-08-010-EZ)

£26,666 - £32,796 p.a.

We are seeking to appoint a Research Associate in Informatics and Data Management to join the Wellcome Trust Centre for Human Genetics, working with Dr. Eleftheria Zeggini. You will be appointed as part of the arc-funded arcOGEN consortium and will contribute to nation-wide efforts to map osteoarthritis susceptibility genes through a large-scale (~8,000 cases) genome-wide association scan. You will have overall responsibility for the informatics and data management of this high-profile project, including integration with publicly available bioinformatics data. We are looking to appoint someone with expertise in informatics, large-scale data management and web development skills. This position may be offered on a part-time basis.

Post four (Ref: H5-08-011-EZ)

£26,666 - £32,796 p.a.

We are seeking to appoint a Postdoctoral Research Associate to join the Wellcome Trust Centre for Human Genetics, working with Dr. Eleftheria Zeggini on empirical and analytical approaches to the design, analysis and interpretation of large-scale association studies. You will work with Dr. Zeggini and her group to address critical questions of large-scale association analysis design and apply the inferences gained to the genome-wide association and resequencing datasets at our disposal. We are looking for someone with a strong record of academic achievement including a relevant degree; programming skills are highly desirable as is experience of working with large-scale association data.

Post five (Ref: H5-08-016-AM)

£26,666 - £32,796 p.a.

You are invited for the post of Research Fellow in the Bioinformatics and Statistical Genetics Group at the Wellcome Trust Centre for Human Genetics. You will work with Dr. Andrew Morris on the design and analysis of data from whole genome association studies. The position offers an exciting opportunity for a scientist to have hands on experience of working with genetic data from large-scale whole genome association studies, including that obtained as part of the Wellcome Trust Case Control Consortium. You will be responsible for the development of methodology for the analysis of whole genome association studies, focusing on epistasis and gene-environment interactions and haplotype analysis. You should have a PhD in statistics, computing or biology or a related subject, experience of the application of statistical methods to genetic data and expertise in scripting and/or programming languages and statistical software packages. Previous experience in the analysis of genome-wide association scans for complex human disease would be an advantage, but is not essential.

All positions are available between 2- 4 years in the first instance. Further information on how to apply for these positions is available from the Personnel Administrator, e-mail: personnel@well.ox.ac.uk or tel. (01865) 287508 or from our web page: www.well.ox.ac.uk/vacancies Please quote the appropriate reference number for the post(s) you are interested in. The closing date for applications is 7 March 2008.

As an Equal Opportunity employer, we positively encourage applications from people of all backgrounds

U125213RM

www.ox.ac.uk/jobs

Senior Lab Technician
Lab Technician needed
Applicant must provide technical and administrative support to R&D projects for evaluating various post harvest technologies for fruits, vegetables, and flowers, execute experiments and routine fruit quality measurements, set-up, operate and maintain lab instruments & preparation of graphs, tables and report writing. B.S or M.S in Biology required.

Contact
http://www.kellyscientific.com
NW125006RL

Associate Director
Needed for Analytical Services
Applicant must develop, validate, and utilize bioanalytical LC-MS/MS and conventional HPLC methods for the quantitative analysis of small molecule drugs and their metabolites in plasma, other biological matrices and animal dosing formulations in support of GLP toxicology and clinical PK studies. PhD in Chemistry needed.

Contact
http://www.kellyscientific.com
NW125007RL

Research Scientist
Hire Opportunity - Memphis
Candidate is responsible for conducting/overseeing studies to examine the solubility, stability, pre-formulation, dosage form development, and other physicochemical properties of proprietary drug candidates and will closely work with medicinal chemists to characterize pharmaceutical ingredients. Must have Ph.D. in physical chemistry or related.

Contact
http://www.kellyscientific.com
NW125010RL

Manufacturing Technician
Biotech/Pharmaceutical
The manufacturing technician is responsible for executing all processes in production, must receive and distribute supplies into the production area, and must participate in both departmental projects and any quality working teams which may be applicable. Candidate must have AA or high school diploma with 1-2 years related manufacturing experience.

Contact
http://www.kellyscientific.com
NW125011RL

Science Recruiter
Staffing Company opportunity
Scientific Staffing Company has an opportunity for a key individual who will drive the recruiting function for the branch. BA/BS in Chemistry, Biology or science-related field required. Prefer 1-2 years experience working in a laboratory/scientific setting recruiting. Must have strong interpersonal, customer satisfaction, and leadership skills.

Contact
http://www.kellyscientific.com
NW125012RL

Biology Laboratory Technician
Microbiological/Molecular Bio
RESPONSIBILITIES include:
- Conducting laboratory experiments
- Develop upcoming research projects
- Compile, analyze and document experimental data and results

REQUIREMENTS include:
- Bachelor's Degree in the Biological Sciences
- At least one year of experience utilizing laboratory techniques within the biological sciences

Contact
http://www.kellyscientific.com
NW125013RL

QA Supervisor
Food Manufacturing Facility
Responsibilities include supervision of second shift QA compliance activities, coordination of QA procedures and programs, document control coordination and training activities, and resolving quality issues related to laboratory testing of product samples. Qualified candidates will possess BS/BA in Scientific discipline.

Contact
http://www.kellyscientific.com
NW125014RL

Entry Level Quality Analyst
Biotech company
Education: Minimum of Associates Degree in the physical sciences.
Experience: Computer skills, strong knowledge of Excel. Mechanical aptitude
Job Function: Collect production and test data from various sources and enter into an Excel spreadsheet. Assist with and perform data analysis. Collect and prepare membrane samples for testing. This position will not be in a laboratory setting.

Contact
http://www.kellyscientific.com
NW125015RL

Software Validation Engineer
Medical Device Company
Contract is for 1 year. Leading medical device company in south Dublin has an opening for a Software Validation Engineer. They are looking to fill immediately. This is an excellent opportunity to gain valuable industrial experience with a market leader. Requires third level qualification in relevant area of Engineering/Quality or Manufacturing.

Contact
http://www.kellyscientific.com
NW125016RL

Analytical Chemist
Leading pharmaceutical company
This position's key function is to provide analytical support to Research and Development and to perform analytical chemistry work, and is responsible for developing new analytical methods in conjunction with process development research. Must have B.S. in Chemistry or related and minimum of 5 years HPLC experience.

Contact
http://www.kellyscientific.com
NW125017RL

Technical Chemist
Chemical Manufacturing
A great opportunity for candidates looking for part time work!
Seeking a Technical Chemist to perform wet chemistry analysis, trouble shooting and some technical service work. The company is small/medium enterprise with a down-to earth and friendly culture. Permanent Role - 20 hr. week Mon thru Fri -Salary £7.69-8.65/h
Ref - p1070aw

Contact
http://www.kellyscientific.com
NW125018RL

Packaging Technician
Adhesive Manufacturing
Dublin West plant. Main responsibilities will include:
- To make available results of technical activities;
- To observe secrecy requirements of the company;
- To perform testing on packaging materials and products;
- To carry out evaluation of new packs and moulded components;
- To perform testing and qualification of new packaging technologies.

Contact
http://www.kellyscientific.com
NW125020RL

Environmental Scientist
Environmental Company
Expected to have a minimum of a third level degree or higher in environmental science, environmental engineering or another appropriate discipline with at least three years relevant industry experience. Must have an in-depth knowledge of a broad range of environmental issues, and have practical experience in environmental monitoring.

Contact
http://www.kellyscientific.com
NW125070RL

Marketing Manager - Polymers
High quality polymers & resins
You will lead and be very market driven. With the ultimate goal of expanding the business across Europe the role will encompass PR, Advertising, Market Segmentation, Website and interaction with other departments. The candidate will have significant experience of marketing within the chemical industry and a post graduate diploma in marketing.

Contact
http://www.kellyscientific.com
NW125072RL

Senior Regulatory Affairs Officer
Regulatory Affairs Department
The candidate will ensure compliance with CE marking and all other appropriate regulatory requirements; Regulatory Registrations; Labelling; Post Market Surveillance and Vigilance; Clinical and Biological Evaluations; Providing Regulatory input to Design and Process Change projects. Must have a Degree in Life/Biomedical Sciences or Chemistry.

Contact
http://www.kellyscientific.com
NW125073RL

Software Validation Engineer
Medical device company
RESPONSIBILITIES: execution of software validation protocols; diving and coordinating software validation activities across multiple manufacturing functions; Production of Software Validation/21 CFR Part 11 metrics; 21 CFR Part 11 assessments and remediation planning. Must have third level qualification in Engineering.

Contact
http://www.kellyscientific.com
NW125074RL

Drug Regulatory Affairs Manager
Regulatory affairs support
You will be responsible for the management of Health Authority interactions in the European Union. ou will prepare regulatory submissions and you will review and/or write Module 2 documents. You participate in the implementation of an electronic Document Management System. You have a scientific degree in Pharmacy, Chemistry, Biology.

Contact
http://www.kellyscientific.com
NW125075RL

Data Management Specialist
Pharmaceutical Company Royston
Candidate must ensure timely entry of all product and process data and ensuring products produced to correct requirements, monitor data and alert management if problems arise, and assist in preparation of operating procedures for inputting and maintaining data. Must have A level Maths and ideally hold a recognised Statistics Qualification.

Contact
http://www.kellyscientific.com
NW125076RL

Scientific Director
Cosmetic Dermatology
Reporting to the CEO of the company, you will coordinate technical and human resources of the Scientific Department, ensure the action plan continuity and the products viability. And to define and implement policy, to coordinate development, and to manage a team of technicians to increase efficiency. Must have Scientific PhD (preferably Chemistry.)

Contact
http://www.kellyscientific.com
NW125077RL

Senior Scientist
Hire Opportunity - Memphis
Candidate will be conducting HPLC and HPLC/MS bioanalytical method development, validation, and sample processing to support drug discovery/development programs, and interacting with preclinical scientists conducting in vitro and in vivo pharmacology experiments to discover and characterize novel molecules. MS or PhD in analytical chemistry needed.

Contact
http://www.kellyscientific.com
NW125009RL

To view these positions and more, log on to http://www.nature.com/naturejobs/profiles/kelly.html

Chair
NC3Rs Board

National Centre for the Replacement, Refinement and Reduction of Animals in Research

The NC3Rs is a scientific organisation at the forefront of applying the 3Rs (Replacement, Reduction and Refinement) to the use of animals in research in the biosciences. Funded by the Government and the private and charitable sectors, and sponsored by the Department for Innovation, Universities and Skills (DIUS), the Centre has an annual budget of approximately £3.5 million. The Centre's strategy includes supporting high quality research, improving access to information and resources and establishing networks and partnerships to facilitate the implementation of the 3Rs in academia and industry.

The NC3Rs engages with a wide group of stakeholders including scientists, veterinary and animal care staff, regulators, research funding bodies, parliamentarians, policy makers, animal welfare groups, the general public and the media.

Lord Turnberg of Cheadle has retired as Chairman of the NC3Rs Board and a successor is now being sought. The ideal candidate will be a highly respected individual with a proven track record in the scientific community, have an excellent understanding of the 3Rs, command the respect of the partners and stakeholders of the NC3Rs, and contribute effectively in a non-executive capacity to organisational policy, strategy and financial planning.

Further information, including an application form can be obtained from recruit@nc3rs.org.uk

Applications should arrive no later than 6 March 2008. Interviews will be held on the morning of the 18 March 2008.

Appointments to the NC3Rs Board are made in accordance with the principles of the Code of the Commissioner for Public Appointments. The Board normally meets three times a year and an honorarium is paid for attendance.

Further information on the NC3Rs including the most recent Annual Report can be found at www.nc3rs.org.uk

Using the 3Rs to support science, innovation and animal welfare

U125184R

kmc international

bbsrc biotechnology and biological sciences research council

CHIEF EXECUTIVE
Biotechnology and Biological Sciences Research Council

The Secretary of State for Innovation, Universities and Skills wishes to appoint a Chief Executive of BBSRC to lead the Council in the pursuit of its mission.

Candidates need to be able to command the respect of the academic and user communities across the domain of biological sciences through their personal achievements in research and their standing in the field. They will also need to demonstrate the ability to provide sound management and strong leadership of a substantial enterprise with efficiency and probity, combined with the personal skills required to represent the Council and foster links with its numerous and diverse stakeholders across Government, academia, learned societies and industry, both in the UK and internationally.

A salary will be available commensurate with the level of seniority and responsibility of the post. The appointment will be full time and based in the BBSRC's offices in Swindon although considerable travel within the UK will be required. The post is offered for a fixed term of four years, with the possibility of renewal.

The Secretary of State appoints members to Councils on merit after considering advice from an Appointments Panel. The Secretary of State is committed to the principle of public appointment with independent assessments, openness and transparency of process and to providing equal opportunities for all, irrespective of race, age, disability, gender, marital status, religion, sexual orientation, transgender and working practices.

U125197R

Further information including details of how to apply can be obtained from DIUS' retained recruitment consultants, kmc international, at www.kmcinternational.com or by e-mail from bbsrc@kmcinternational.com or by telephone on +44 (0)2920 353383. Please quote reference 1294/1.

The closing date for applications is Friday 29th February 2008.

Chief Scientist

Sustainable Forestry Management Ltd (SFM) is a leading global investor in subtropical and tropical forests on an integrated, sustainable and ethical basis. SFM invests in partnership with the private, public and non-governmental sectors to provide sustainable livelihoods in restored and conserved forest ecosystems. SFM is building a global portfolio of forest land assets and becoming a leading supplier of certified carbon and other environmental credits and offsets. SFM is active in Africa, Latin America, Asia and Australasia.

SFM is seeking a Chief Scientist with a strong background in environmental science to join its senior executive team. The successful applicant will have significant practical experience in conservation ecology as well as a strong record of academic achievement. Extensive knowledge of tropical and subtropical forest areas is required. Experience in the forestry, agro-forestry and horticultural sectors is desirable, together with working knowledge of Spanish, French and/or Portuguese in addition to fluent English.

Qualifications

- PhD or equivalent degree, and a record of peer reviewed first author papers
- Global network of scientific contacts in the fields of tropical and sub-tropical botany, biology, ecology and related climate science

Experience

- Building R&D programs with proven conservation, commercial and land management impacts in the developing world
- Substantial international multidisciplinary field experience in tropical and sub-tropical forest areas
- Senior management experience in commerce, the public sector or academia

Skills

- Proven leadership skills to build and inspire an R&D ethos in a market-driven environment
- Proven senior management and administrative skills
- Lateral thinker capable of finding new approaches to sustainable land use
- Experience of working with multi-disciplinary teams
- Strong and confident communication skills

Ideally, this position will be located in London. There is some flexibility but overseas applicants must be eligible to work in the US and/or UK and to travel extensively.

Excellent remuneration and benefits package commensurate with experience and skills.

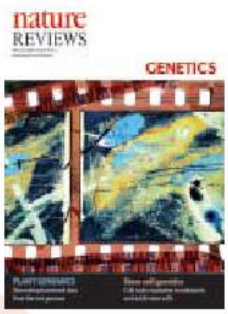
Please send CV and cover letter to PAtoebettelheim@sfm.bm

Further information about SFM can be found at **www.sfm.bm**

W124893R

nature REVIEWS

Chief Editor Position



Nature Reviews Genetics - the most highly-cited monthly review journal in genetics and heredity - is seeking to appoint a new Chief Editor with the remit to further develop the journal's coverage and impact. This is a challenging, exciting and rewarding role with overall responsibility for commissioning and editing reviews, managing the editorial team, representing the journal at international conferences and planning special issues and projects.

The ideal candidate will have a PhD and post-doctoral experience in a relevant subject area and a broad interest in genetics and genomics. Although desirable, previous publishing experience is not essential; however, candidates should have genuine enthusiasm for science communication and an understanding of the editorial process. Other important attributes include a dynamic, outgoing personality and good team-working skills. The position includes line-management responsibility so people management experience is also desirable.

The position is based in Nature Publishing Group's modern London offices, and the terms and conditions are highly competitive, reflecting the critical importance and responsibilities of the role. For further information about *Nature Reviews Genetics* please visit http://www.nature.com/nrg. Applicants should send a CV and a brief cover letter explaining their interest in the post, quoting reference number NPG/LON/806 to: Denise Pitter, Personnel Assistant, Macmillan Publishers at londonrecruitment@macmillan.co.uk

Closing Date: Monday 18th February 2008

nature publishing group npg

IN124915R

Leeds Institute of Molecular Medicine

UNIVERSITY OF LEEDS

Faculty of Medicine and Health

Genotype-Specific Therapeutics and Chemical Biology

Postdoctoral Research Fellow

Postdoctoral Research Fellow to work in the group of Terence Rabbitts (collaborative with Colin Fishwick & Peter Johnson, School of Chemistry)

Applications are invited for a postdoctoral position in Leeds Institute of Molecular Medicine (LIMM) to work on computer-aided molecular design and synthesis of new inhibitors of mutant proteins resulting from cancer-specific mutations. The post will be held in LIMM but the work will be in close collaboration with Dr. Colin Fishwick and Professor Peter Johnson in the University of Leeds, School of Chemistry.

Terry Rabbitts (Director of LIMM) is based at the St. James's University Hospital site, in the new Wellcome Trust Brenner building and Peter Johnson and Colin Fishwick in the School of Chemistry on the University of Leeds campus. LIMM has core facilities for molecular and cell biology research and the School of Chemistry is well equipped for synthetic chemistry, and includes facilities for NMR, mass spectrometry and computing for *in silico* design.

The postholder will design and prepare small chemical molecules (in the School of Chemistry) that bind to oncogenic proteins and affect their function. The target proteins will be ones mutated in human cancers either due to chromosomal translocations or due to point mutations. Lead compounds will be developed by medicinal chemistry and efficacy tested at all stages in collaboration with the biologists in LIMM.

The post is available immediately for a fixed term of three years at **University Grade 7 (£27,466 - £32,796 p.a.) depending upon qualifications and relevant research experience**

Applications should include a covering letter outlining the reason for the interest in the post, a CV and the name of three academic referees from whom immediate references may be obtained. The full job specification must be viewed online at http://www.leeds.ac.uk/medhealth/limm/vacancies.html Applications should be made by email to Mrs Jennifer Flowerdew j.h.flowerdew@leeds.ac.uk or tel +44 (0)113 343 8441

Job ref 314185 Closing date 13 March 2008

We welcome applications from all sections of the community. Textphone for deaf applicants only +44 (0)113 343 4353. All information is available in alternative formats please contact +44 (0)113 343 4146.

U125187R

WORKING TOWARDS EQUALITY AND DIVERSITY www.leeds.ac.uk

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www.cam.ac.uk/jobs/

A world of opportunities

UNIVERSITY OF CAMBRIDGE

Research Associate

Department of Veterinary Medicine
Salary: £25,134 - £32,796 pa
Limit of tenure: 3 years

We require a Research Associate to work on an MRC funded project examining the effect on functional outcome of intraspinal cell transplantation into severe clinical spinal cord injuries in dogs.

You will have skills in cell culture, gait analysis and a willingness to handle dogs. Experience with matrix analysis software and clinical work would be advantageous.

Applicants should have a PhD in a related subject area and ideally have experience in managing projects.

For further information about the role contact Professor Nick Jeffery (ndj1000@cam.ac.uk or 01223 339969) or Professor Robin Franklin (rjf1000@cam.ac.uk or 01223 337642).

Further particulars are available at: http://www.vet.cam.ac.uk/news/

- **Applicants should supply the following documents:**
- **A letter of application stating areas of interest**
- **A full Curriculum Vitae, with the names and contact details of three referees**
- **A completed application form PD18, (parts one and three only) available from the Melissa Large on 01223 337055 or from: www.admin.cam.ac.uk/offices/personnel/forms/pd18/**

Applications should be sent for the attention of Miss Melissa Large, Department of Veterinary Medicine, Madingley Road, Cambridge, CB3 0ES. Applications can be made via email to recruit@vet.cam.ac.uk with the above documents as word attachments.

Please quote reference: PN03013.

Closing date: 6 March 2008. Interview date: 18 March 2008.

The University is committed to Equality of Opportunity.

U125204R

Max Planck Institute for Neurological Research

MAX-PLANCK-GESELLSCHAFT

with Klaus-Joachim-Zülch-Laboratories
of the Max Planck Society and the Faculty of Medicine
of the University of Cologne
Director Prof. Dr. D. Yves von Cramon

University of Cologne

Medical Faculty
Dean Prof. Dr. Joachim Klosterkötter

The Max Planck Institute for Neurological Research invites applications for a Group Leader position

Selbständige Nachwuchsgruppe der Max-Planck-Gesellschaft (Independent Junior Research Group)

The Max Planck Institute for Neurological Research and the Medical Faculty of the University of Cologne has opening for a Group Leader position, funding for a research group (Selbständige Nachwuchsgruppe der Max-Planck-Gesellschaft / Independent Junior Research Group) dedicated to neurological and oncological research. While there is no particular restriction on the research area, there is the expectation that the successful candidate will interact synergistically with other groups at the Institute.

Currently, the following technologies / methods are available at the Institute: **human brain PET and MRT, animal PET and MRT, optical imaging, autoradiography, electron microscopy, histology, immunohistochemistry and invasive imaging / neuromonitoring.** A **combined human brain PET-MR System** will be installed.

Cologne and its rich scientific environment in North-Rhine-Westphalia provide many opportunities of interactions with the University of Cologne and the Universities in the region, including the teaching of courses.

The Group Leader position and funding is guaranteed for five years with the possibility of extension.

The Max Planck Society and the University of Cologne aim to increase the representation of women and therefore explicitly encourage applications from female scientists.

Deadline for applications is March 28, 2008. For further details and the application procedure please visit www.nf.mpg.de/careers.

W124899R

Nycomed is a pharmaceutical company that provides medicines for hospitals, specialists and general practitioners, as well as over-the-counter medicines in selected markets.

The company is active within a range of therapeutic areas, including cardiology, gastroenterology, osteoporosis, respiratory, pain and tissue management. New products are sourced both from own research and from external partners. Operating throughout Europe and in fast-growing markets such as Latin America, Russia/CIS and the Asia-Pacific region Nycomed has a presence in about 50 markets worldwide.

Within Technology & Support, a department of Early Discovery, we are looking for a

Head of Early Discovery Technology and Support f/m

Code 7224*

The ideal candidate should be a senior scientist to lead the technology and support unit within our Early Discovery unit. Core responsibilities of our integrated Early Discovery unit span from Early Target Identification until nomination of Lead Candidates. The unit is supported by our chemistry units in Mumbai and Konstanz and our pharmacology unit near Hamburg.

Responsibilities of this position include supervision of MTS and HTS operation, high content imaging, Discovery IT services, molecular modeling and eADMET profiling of compounds. You will be a member of the Early Discovery leadership team, will head a team of about 15 scientists and are expected to contribute with our team to Lead Discovery projects and to shape the unit with respect to technological approaches.

You should have a PhD in Physics, Biophysics or Biochemistry, several years of professional experience in the industrial drug discovery field and an in-depth understanding of science at the interface of biology, chemistry and physics. Hands-on experience in one or several of the relevant fields would be an additional plus. A proactive and open personality, the ability to work in cross-functional teams, excellent organization, leadership and communication skills are key. Fluent English and German are required.

We offer a challenging position in a professional environment with a very good team spirit. You will have good opportunities to develop your professional as well as personal competencies via on-the-job and off-the-job training. We will inform you in detail of the related responsibilities, our contractual arrangements and attractive benefits (support in obtaining accommodation, reimbursement of removal costs, a competitive salary and a company pension scheme).

Are you interested? Please apply online referring to Code 7224* under www.nycomed.com/de/Menu/Careers.

Please let us know your salary expectations and earliest entry date.

NYCOMED GmbH
Human Resources Germany
Byk-Gulden-Strasse 2, D-78467 Konstanz
Tel. ++49 7531/84-2422 – Jutta Stader
www.nycomed.com

W125091R

Kyoto University Pioneering Research Unit for Next Generation
1 Faculty Position in Organoelement Chemistry at the Assistant Professor Level

Kyoto University Pioneering Research Unit for Next Generation invites applications for a full-time appointment at the Assistant Professor level as a Program-Specific Fixed-Term Faculty member in Organoelement Chemistry. Kyoto University Pioneering Research Unit for Next Generation is in part supported by a five-year Special Coordination Fund for Promoting Science and Technology (April 2006 to March 2010) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT).

For more details, visit:
http://www.kyoto-u.ac.jp/english/enotice/e05_sako/AssistantProf/080229_4.htm

Please be sure to visit the above URL to download an application form and to obtain necessary information for your application. (***deadline: Feb. 29, 2008***)

Kyoto University
Pioneering Research Unit for Next Generation

JP124440R

Max Planck Institute of Microstructure Physics

Director of the Theory Department

(succession of P. Bruno)

We seek an experienced theoretical physicist working in the field of nanoscale solid state phenomena, possibly covering magnetism, electronic transport, ferroelectricity or photonics. Ideally, she or he likes to work analytically, complemented by density functional and dynamical mean field theory calculations.

The Institute is part of the Max Planck Society, comprising the theory department and two experimental departments, working on nano-magnetism and correlation spectroscopy (headed by J. Kirschner) and on semiconducting, multiferroics and photonic nanostructures (headed by U. Gösele). Our new colleague would experience ideal working conditions and close collaboration with the theoretical physics department of the University Halle-Wittenberg.

The Max Planck Society is interested in increasing the proportion of women among its scientists and strongly encourages women to apply to the corresponding positions. Applications from disabled candidates are encouraged and will be given priority in case of equal qualification.

Please, send your CV and a short statement of your interests until **March 15, 2008.**

If you have further questions, please contact the managing director:

Jürgen Kirschner
Max Planck Institute of Microstructure Physics
Weinberg 2, 06120 Halle, Germany

Phone: ++49(0)345-5582-655
sekrki@mpi-halle.mpg.de

W122462R

Barts and The London
School of Medicine and Dentistry

Centre for Infectious Disease

Postdoctoral Research Assistant

(Ref: 08042/HR)

We are looking for an experienced Postdoctoral Research Assistant to establish genome sequencing using new technologies of varicella-zoster virus DNA recovered from clinical material. The project will examine VZV vaccine strains which are recovered from patients with post-immunisation rashes. Building on previous observations in our group (Quinlivan et al 2007, PNAS 104,208), you will sequence a number of vaccine genomes which have caused rashes and using reverse genetics, reconstruct live viruses carrying the relevant mutations. These viruses will be analysed in epithelial models to determine whether the rash mutations in the vaccine strain confer a replication advantage in skin. The effect of vaccine mutations in the major immediate early ORF 62 protein on the behaviour of early replication events following entry of the virus will be examined. You will work with a research assistant who will help with the high throughput DNA extraction, PCR and sequencing.

You should have a PhD and preferably at least 3 years' post-doctoral research experience. A background in virology, genetics or cell biology is preferred. Experience with any or all of the following: sequencing technologies, cell culture, recombinant DNA technology, and confocal microscopy would be helpful. Further details of the project are available from Professor Judy Breuer, Centre for Infectious Disease (tel: 020 7882 2308, email: j.breuer@qmul.ac.uk).

This is a full-time fixed term post for 3 years funded by the MRC. The starting salary will depend on the level of experience and the responsibility undertaken and will be at the appropriate point for experience on the Research scale (Grade 4/5 - £27,813 - £36,458 or Grade 6 - £38,515 per annum inclusive of London Allowance - see relevant job description for each grade).

Candidates must be able to demonstrate their eligibility to work in the UK in accordance with the Asylum and Immigration Act 1999.

For an application form and further information, please visit the Human Resources website on http://www.hr.qmul.ac.uk/vacancies or request details (quoting the above reference number) via email wch-recruit@qmul.ac.uk

The deadline for return of completed applications is 12 noon (GMT) on 29 February 2008. Applications (quoting the above reference number) should be returned via email to wch-recruit@qmul.ac.uk Alternative means of applying are available; please contact the recruitment line on 020 7882 6109 for details. Please note: completed applications must not be sent directly to the Centre for Infectious Disease, or to Prof Breuer.

Where applicants are short listed for interview, references will normally be taken up prior to interview. Applicants who do not wish any referees to be so contacted should make this explicitly clear on their application. We are unfortunately unable to reply to those applicants who have not been shortlisted and invited for interview. However, we would like to thank all candidates for their applications and for their interest in working for the School of Medicine and Dentistry.

Promoting excellence in teaching, learning and research
Working towards equal opportunities

U125193RM

FACULTY POSITIONS AT THE ROCKEFELLER UNIVERSITY

The Rockefeller University seeks exceptional, interactive, and creative scientists to join its faculty. We invite applications from outstanding candidates for tenure-track positions and also encourage tenured Professors to apply, provided that they are early on in their careers.

The Rockefeller University provides strong financial support for the research work of its faculty. The positions offer highly competitive salary, benefits and start-up funds, new or recently renovated laboratory space, access to numerous state-of-the-art core facilities and extensive opportunities for collaboration both within the University and with neighboring institutions. The University also provides very strong continuing support for research work beyond start-up, including full support for graduate students.

The University has a laboratory-based organization structure that fosters interdisciplinary research in the following areas:

- **Biology of Single Cells**
- **Chemical Biology**
- **Evolution, Ecology, and Developmental Biology**
- **Medical Sciences and Human Genetics**
- **Microbiology and Immunology**
- **Neurobiology and Behavior**
- **Physics and Biology**
- **Physiology of Multicellular Organisms**

Details about specific subjects of research can be found at: **http://www.rockefeller.edu/facultysearch.**

Applications are being accepted electronically through our **Online Application System** at **http://oas.rockefeller.edu.** Applicants should follow the online application procedure which includes submitting the following:

- Curriculum Vitae with a publications list
- Statement of Research with a 2 page description of significant research accomplishments and a 2 page description of future research plans (4 pages maximum)
- Relevant publications (optional, maximum of 3)
- Contact information for at least 3 references for applicants who do not currently have a tenure-track or tenured faculty position

If you have questions regarding submitting an application, please contact our Administrator at **facultysearch@rockefeller.edu.**

The deadline for receipt of applications is April 15, 2008.

The Rockefeller University is an Affirmative Action/Equal Opportunity/ VEVRAA Employer and welcomes applications from women and under-represented minorities.

NW124852R

Faculty Position in Liver Research

change the outcome®

The Division of Gastroenterology, Hepatology and Nutrition and the Liver Research Group of Cincinnati Children's Hospital Medical Center and Research Foundation invite applications for professorship, at either assistant or associate level, for research in liver regeneration or progenitor cell biology.

We seek a candidate with an MD or PhD degree and outstanding prospects for research, who will complement existing strengths of the Liver Research Group in the fields of liver development, regeneration and progenitor cells. A successful applicant must be committed to develop and implement independent, externally funded research programs, mentor students and facilitate interdisciplinary research. The successful applicant will be a member of a highly collaborative group of investigators and will have access to outstanding core resources at the NIH-funded Digestive Health Center and Cincinnati Children's Research Foundation.

Cincinnati Children's Hospital Medical Center and Research Foundation are internationally recognized as one of the nation's top pediatric care and research institutions. The Research Foundation ranks second nationally in NIH funding to full-service children's hospitals. Cincinnati is a friendly, pleasant, affordable city with a great quality of life, including many musical and theatrical programs, professional sports and nearby recreational opportunities.

Applicants should submit a curriculum vitae and statement of research interests to:
Dr. Jorge Bezerra, Director of Research
Division of Gastroenterology, Hepatology and Nutrition
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue, Cincinnati, OH 45229-3039
Jorge.Bezerra@cchmc.org

Cincinnati Children's Hospital Medical Center

Visit our website at www.cincinnatichildrens.org

Cincinnati Children's Hospital Medical Center is an Affirmative Action/Equal Opportunity Institution. Women and minorities are encouraged to apply.

NW123455R

NW124981R

FIU

FLORIDA INTERNATIONAL UNIVERSITY
Miami's public research university

ASSOCIATE DEAN FOR RESEARCH

Florida International University - College of Medicine seeks applications for the Associate Dean for Research. We are seeking a candidate to help the College of Medicine leadership facilitate expansion of institutional research. Responsibilities include hands-on data analysis, interpretation of results for internal and external distribution, and assurance of compliance with regulatory authorities. Ability to work with both basic medical scientists, clinical investigators, physicians and other university academic researchers is essential. The Associate Dean for Research is a 12-month full-time staff member reporting to the Executive Associate Dean for Academic Affairs. The successful candidate will have a distinguished record of investigative and currently funded extramural research that can be transferred to a new venue.

FIU emphasizes research as a major component of its mission. Sponsored research funding (grants and contracts) from external sources for the year 2005-2006 totaled $92 million. The University is ranked as a Research University in the High Research Activity category of the Carnegie Foundation's prestigious classification system.

For information or to apply please visit us online at **http://www.fiujobs.org** and reference position #6004. Please be sure to indicate your interest in Associate Dean for Research position in your cover letter. Application review will begin February 11, 2008. Positions will be open until filled.

FIU is a member of the State University System of Florida and is an Equal Opportunity, Equal Access Affirmative Action Employer.

Founding Chair Neurosciences Department

SCRIPPS FLORIDA
A DIVISION OF THE SCRIPPS RESEARCH INSTITUTE

The Scripps Research Institute soon will dedicate the new permanent facilities on its Jupiter, Florida campus. A unique opportunity exists for a highly-qualified individual to be the founding Chair of the Department of Neurosciences. This individual will build a department that draws on the substantial resources already present at Scripps Florida and funds that are available for recruiting 10–12 new faculty members.

Of special note are the high-throughput molecular screening center, the largest of its kind in academia, and core facilities in genomics, proteomics, and structural biology. The new Department of Neurosciences will complement existing Departments of Cancer Biology, Infectology, Metabolism & Ageing, and Molecular Therapeutics at Scripps Florida, and its faculty will enjoy collegial interactions with the neuroscientists at Scripps in La Jolla. In addition, the Max Planck Society, the State of Florida, and Palm Beach County recently approved plans to establish a Max Planck Institute, the first in the U.S., located adjacent to Scripps Florida. This Institute will focus on bio-imaging, and will be a collaborative partner with the neuroscientists at Scripps Florida.

Candidates should send a letter of application and complete curriculum vitae to Dr. Gerald F. Joyce, Dean of the Faculty and Professor, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037.

TSRI embraces diversity and recognizes it as being a key to our success.
We believe in developing and maintaining a diverse workforce (EOE/M/F/D/V).

NW124874R

nature methods

Locum Assistant Editor

Nature Methods seeks a Locum Assistant Editor to join their editorial team for a period of six months to cover a maternity leave. The journal publishes high quality papers that represent major methodological developments, likely to be influential in the life sciences. In the tradition of *Nature* journals, this selection relies on a thorough peer review process.

For more information about the journal, see our website (**http://www.nature.com/nmeth**).

Members of the editorial team evaluate manuscripts, oversee the peer review process, commission and edit secondary materials such as Reviews, and write short pieces and editorials for the journal. The new editor will join our team in the NYC office of the larger Nature Publishing Group.

Candidates should have a broad interest in science, excellent communication skills, and a willingness and ability to learn new fields. Applicants should have completed a Ph.D. in any of the areas covered in *Nature Methods*.

To apply, please submit a CV, and a cover letter explaining your interest in the position and your possible start date to Human Resources Department, Nature Publishing Group, e-mail: **admin@natureny.com**

Applications should arrive as soon as possible with a *close date of February 28, 2008.*

NPG is an EOE.

nature publishing group npg

IN123698R

100 Top Hospital expanding in Central Texas

TEXAS A&M INSTITUTE OF REGENERATIVE MEDICINE IN TEMPLE, TX.

Post-doctoral and Faculty Positions

The newly established Texas A & M Institute of Regenerative Medicine is seeking post-doctoral fellows and faculty for research on adult stem/progenitor cells. The Institute is dedicated to research both on the basic biology of stem/progenitor cells and their potentials for therapies of human diseases. It will occupy newly renovated laboratories and a series of core laboratories in an expanding academic medical center. Post-doctoral appointments will be for one year with the opportunity to renew for a second and third year subject to performance. Faculty appointments will be in an appropriate academic department and will range in rank from tenure-track assistant professors to tenured full professors depending on qualifications. Faculty appointments will include institutionally-funded salaries, start-up funds, modern laboratory space, and access to graduate students. Salaries and benefits are competitive. Candidates should have excellent verbal skills and a Ph.D. or M.D. degree from a well recognized university.

Before March 1, 2008, please send curriculum vitae, brief statement of research interests, indication of level of appointment sought, and three letters of recommendation to attention of Darwin J. Prockop, M.D., Ph.D., Director, Texas A&M Institute of Regenerative Medicine at email address: Regenerate@medicine.tamhsc.edu. The Texas A&M Health Science Center is an AA/EO Employer.

SCOTT & WHITE

TEXAS A&M HEALTH SCIENCE CENTER COLLEGE OF MEDICINE

NW123456R

STANFORD UNIVERSITY DEPARTMENT OF ANESTHESIA

The Department of Anesthesia in conjunction with the Department of Bioengineering at Stanford University seek applicants for a faculty position at the junior (Assistant Professor or untenured Associate Professor) or senior (Associate or Full Professor with tenure) level on the University Tenure Line (UTL). Applicants are expected to have a doctoral degree in bioengineering, biomedical engineering, molecular biology or a related discipline.

The candidate is expected to develop an outstanding translational research program in biological modeling or computational biology. This is intended to be a broad based search and may encompass individuals with a wide range of expertise. The candidate should have the capacity to apply computational approaches to understanding control modeling of cell signaling, genetic contributions to disease, cell interaction, or expression phenotypes of disease states, and is expected to collaborate with both clinical and basic scientists to enhance ongoing interdisciplinary programs. The successful candidate will be responsible for securing government and industry funding to support and conduct innovative research.

The overriding requirement for faculty appointment, reappointment and promotion within the UTL must be distinguished performance, or (in the case of junior faculty) the promise of distinguished performance. There should be a major commitment to research and teaching.

Stanford University is an equal opportunity employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups, as well as others who would bring additional dimensions to the university's research, teaching and clinical missions.

Interested candidates please submit curriculum vitae to Dr. Ronald G. Pearl, Chair, Department of Anesthesia, Stanford University School of Medicine, Stanford, California 94305-5640.

NW124783R

Postdoctoral Scientist or Research Associate

Position available to study the molecular genetics of kidney diseases in humans and mouse models. The projects will employ state-of-the-art genomics and bioinformatics approaches, such as genetic linkage and association analyses, and gene expression studies in well-characterized cohorts to identify susceptibility genes for kidney failure. Requires prior training in molecular biology or molecular genetics and bioinformatics.

Please send curriculum vitae and names of three references to Dr. Ali Gharavi, Columbia University, Department of Medicine, Box 84, 630 W. 168th St., New York, NY 10032 or e-mail to: ag2239@columbia.edu.

Columbia University is an equal opportunity/affirmative action employer.

NW124853R

POSTDOCTORAL FELLOW RESEARCHER

Blood Systems Research Institute (BSRI) an affiliate of the University of California in San Francisco, CA seeks a full-time post-doctoral fellow to participate in human translational research on an immune tolerance model, transfusion-associated microchimerism. Transfusion in the setting of trauma results in long-term persistence of donor cells in some blood recipients, and the project will define the mechanisms of this persistence, with broader implications for understanding immune tolerance. Experience with cell culture and flow cytometry preferred.

Candidates should possess a Ph.D., M.D. or both and have a strong background in immunology or virology. Send CV and professional references by 2/15/08 to: Human Resources, Blood Systems Research Institute, 270 Masonic Ave., San Francisco, CA 94118. Fax (415)775-3859; E-Mail: BSRIcareers@bloodsystems.org

Pre-employment drug screen required. EOE M/F/D/V.

NW124849R

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naturejobs making science work

Visit
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naturejobs making science work

MRC Medical Research Council

MRC Cognition Brain Sciences Unit (CBSU), Cambridge

Senior research post in the Cognitive Neuroscience of Memory & Learning

An MRC Programme Leader position is available to create and direct a long-term research programme in the cognitive neuroscience of human memory and learning. Preference will be given to programmes that link to current Unit strengths in neurodegeneration and neurorehabilitation, or to new initiatives in the neuro-cognition of ageing. Excellent candidates in related areas will also be considered. The CBSU houses on-site, fully research-dedicated 3T MRI, MEG and EEG facilities, to which you will have full access. There are also strong links with groups in the University of Cambridge Clinical School at the nearby Addenbrooke's hospital.

The position comes with post-doctoral and research assistant support, and potential for PhD studentships. You should have a strong track record in cognitive neuroscience, neuropsychology or neurorehabilitation, with expertise in neuroimaging. You will be an internationally prominent scientist, with the ability to propose novel theories and experiments, to attract funding, to manage high-quality staff, to work in a co-operative research environment and to think strategically.

The starting salary for this position is negotiable depending upon experience. This is supported by a flexible pay and reward policy, 30 days annual leave and an optional MRC final salary pension scheme.

For an informal discussion, contact William Marslen-Wilson, CBSU Director, by email: william.marslen-wilson@mrc-cbu.cam.ac.uk

Further information on the unit can be found at http://www.mrc-cbu.cam.ac.uk

Applications for this role must now be made online at http://jobs.mrc.ac.uk inputting reference number CBSU08/076. Please ensure that you upload a current CV and covering letter with your application, and include the contact details of three professional referees who can be contacted prior to interview. If you do not have internet access or experience technical difficulties, please call (+44) 1793 301158.

Closing date: 25 March 2008.
Initial interviews will be held in the week of 12 May 2008.
Potential starting date of appointment: September 2008.

For further information about the MRC visit www.mrc.ac.uk

The MRC is an Equal Opportunities Employer

'Leading science for better health'

U125196R

University of Oxford

Wellcome Trust Centre for Human Genetics

5 x Post Doctoral Research Scientists in Statistical Genetics

£33,779 - £40,335 p.a (depending on qualifications & experience)
£26,666 - £32,796 p.a. (with an appropriate change of duties)

An exciting opportunity has arisen for 5 post doctoral scientists in statistical genetics to work on one of two cutting-edge projects in human genetics.

Genome-wide association studies (GWAS) have revolutionised our understanding of the genetics of common human diseases. The Wellcome Trust Case Control Consortium (WTCCC), chaired by Professor Peter Donnelly, was the largest of the first generation of these studies. Together with subsequent collaborations and follow-up, it has led to the discovery of over 50 genetic variants associated with common diseases. *Science* picked this field as its "Research Breakthrough" of 2007 and the WTCCC study won several awards: from *The Lancet* (Research Paper of the Year); *Scientific American* (Research Leader of the Year); *The American Heart Association* (Top Research Advance of the Year) and was one of *Nature's* "Editor's Picks" for 2007.

Posts 1 and 2 relate to the second phase of the WTCCC, a major extension of the original study, which includes association studies for copy number variation in eight diseases and resequencing and fine-mapping of associated loci across 13 diseases. You will work in a collaboration between the Donnelly, Holmes, McVean, Marchini and Myers groups. Posts 3-5 relate to a major new consortium funded by the Wellcome Trust for GWAS for 15 diseases. They will be based in the Donnelly group, which will take primary responsibility for methods development and analysis for these new studies, with extensive collaborations with the groups above.

You will gain a unique insight and experience in the design and analysis of GWAS and high-throughput genetic epidemiological studies. Alongside the scientific investigation you will be given the opportunity and encouragement to follow independent research lines and methods development, with support from the PIs, in the context of statistical genetics.

Genetics is one of the most exciting and challenging application areas in modern statistics. These posts present a good opportunity for statisticians interested in moving into this field, as well researchers already working in statistical genetics and related areas. The Wellcome Trust Centre for Human Genetics is at the forefront of this field and you would join perhaps the leading international research grouping in the area, with a focus both on the development of new methods and their application. All the posts will allow considerable individual involvement and responsibility for the research undertaken.

You should have a strong background in modern statistics and its application. An existing background in the application of statistics in genetics would be an advantage, but candidates wishing to move into the genetics field are also encouraged to apply. Good computational skills are essential and for several of the posts you should be able to program in a low level language such as C or C++. You should have a doctorate or equivalent experience in quantitative genetics, statistics, or a closely related discipline. Posts 1 and 2 are available for 2-3 years and posts 3-5 for 3 years in the first instance. All posts are funded by the Wellcome Trust.

A detailed job description quoting reference H5-08-18-PD is available from the Personnel Administrator, (e-mail: personnel@well.ox.ac.uk tel. +44 1865 287508 or from our web page: www.well.ox.ac.uk/vacancies). Applications, in the form of a full and detailed CV together with the names and addresses of two referees, should be sent to the Personnel Administrator at the Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN, or by e-mail to: personnel@well.ox.ac.uk or fax tel. +44 1865 287516. Please quote the reference number on your application. The closing date for applications is 7 March 2008.

As an Equal Opportunity employer, we positively encourage applications from people of all backgrounds

U125208R

www.ox.ac.uk/jobs

Don't miss the intoxicatingly good job opportunities in *Nature* each week and on naturejobs.com

naturejobs

www.cardiff.ac.uk/jobs

CARDIFF UNIVERSITY

PRIFYSGOL CAERDYDD

Research Associate

Cardiff School of Biosciences

A new position exists for a post-doctoral scientist to join a recently established and rapidly growing laboratory within the Genetics Group at Cardiff School of Biosciences, as part of the BBSRC-funded project: "Nucleosome positioning factors and DNA double-strand breaks".

Studies will focus on elucidating the mechanisms of a variety of chromatin-remodelling processes relating to DNA break repair and recombination. You will gain experience and training in state-of-the-art chromatin analysis techniques using budding yeast as a model system.

The position is fixed-term for 36 months.

Salary: £27466 - £32796 per annum

Informal enquiries can be made to Dr Nick Kent at KentN@cardiff.ac.uk

To work for an employer that values and promotes equality of opportunity, visit www.cardiff.ac.uk/jobs telephone + 44 (0) 29 2087 4017 or email vacancies@cardiff.ac.uk for an application form quoting vacancy number 112.

Closing date: 6 March 2008.

U125206R

University of Oxford

Charles Simonyi Professorship in the Public Understanding of Science

Applications are invited for the above post, tenable from 1 October 2008, or as soon as possible thereafter.

You will be expected to make important contributions to the public's understanding of science. The starting point is likely to be a specific field of science, which may, as well as the natural, medical and mathematical sciences, include the history of science and the philosophy of science.

The professor should be committed to using the opportunities the chair provides through writing, broadcasting and other forms to encourage the public to share in the excitement of science, and foster an awareness of the contribution it can make to human endeavour. You will take a leading role in developing the science outreach activities of the University of Oxford.

You will be expected to continue his or her own research and scholarly work, which may include investigations of the public's understanding of science.

The professorship will be held jointly between the Department for Continuing Education and the appropriate discipline-based department in the University, depending on the postholder's own background.

A non-stipendiary Professorial Fellowship at New College is attached to the professorship.

Further particulars, including details of how to apply, are available from http://www.admin.ox.ac.uk/fp/ or from the Registrar, University Offices, Wellington Square, Oxford OX1 2JD, UK (Tel: +44(0) 1865 270200). The closing date for applications is 31 March 2008.

The University are Equal Opportunity Employers. We positively encourage applications from people of all backgrounds

U125209RM

www.ox.ac.uk/jobs

College of Life Sciences and College of Medicine, Dentistry and Nursing

THE CHAIR OF TRANSLATIONAL MEDICINE

We are combing the world for an exceptional leader in translational medicine to head our first class team of clinicians and scientists in this vital and dynamic field. You will be a scientist or clinician already working at the level of professor, with an international track record in scientific achievement related to one of our key strengths: pharmacogenetics, cancer, inflammation, neurosciences, cardiovascular disease or diabetes/metabolism.

This new University-funded Professor of Translational Medicine will provide scientific leadership in disease-related therapeutic areas, working closely with scientific teams to develop biomarker discovery programmes and support the application of translational medicine to therapeutic trials. This strategic appointment will be made jointly between the internationally acclaimed College of Life Sciences and the College of Medicine, Dentistry and Nursing, and is associated with a very substantial start up package. The University is well funded in this area and is superbly equipped with state of the art technologies to study translational medicine.

You will work with strong and diverse teams, managing multiple projects and interacting with the Translational Medicine Research Collaboration (www.tmrc.co.uk), a unique £50m pan-Scotland research partnership in collaboration with Wyeth. An £11m dedicated biomarker laboratory will open in Dundee in October 2008.

For more information about this outstanding opportunity to play a key leadership role in one of the most ambitious programmes in translational medicine globally, and application instructions contact: Professor Irene Leigh, Vice Principal and Head of College of Medicine, Dentistry and Nursing; i.m.leigh@dundee.ac.uk; or Professor Peter Downes, Vice Principal and Head of College of Life Sciences, University of Dundee; c.p.downes@dundee.ac.uk

Interviews are likely to be held February/March 2008.

Further details and an application pack are available from our website: www.jobs.dundee.ac.uk Alternatively, contact Personnel Services, University of Dundee, Dundee DD1 4HN, tel: (01382) 344817 (answering machine). Please quote reference number: LS/2092/N.

As part of the recruitment process, the University requires that a Disclosure Scotland check is undertaken for this position.

Closing date: 8 March 2008.

The University of Dundee is committed to equal opportunities and welcomes applications from all sections of the community.

U125201R

www.dundee.ac.uk/jobs

UNIVERSITY OF DUNDEE

UCL

UCL The Ear Institute

Senior Research Associate - British Tinnitus Association

The UCL Ear Institute is a multi-disciplinary facility which opened in January 2005 with the remit of "understanding hearing and fighting deafness". In collaboration with the British Tinnitus Association, the Institute is looking to recruit a new investigator for a Senior Research Associate position, available from June 1st 2008, funded for an initial period of three years with the possibility of further funding for an extension up to five years.

As part of the British Tinnitus Association's "pill for tinnitus" campaign, the Investigator, reporting to the Director, will be expected to conduct an independent (but interactive) research programme within the UCL Ear Institute investigating physiological and/or pharmacological aspects of central auditory processing and their relationship to tinnitus.

The successful applicant will be given support and encouragement to obtain external funding during the term of the appointment. The focus of this particular search is for outstanding individuals whose expertise lies in one or more of the following fields of endeavour: electrophysiology including cochlear physiology, neuroscience of the central auditory system including neuropharmacology and auditory modelling, and animal behaviour.

Salary will be on Grade 8 of the UCL Salary Scale which ranges from £34,793 - £41,545 per annum plus £2,649 London Allowance per annum.

Informal email enquiries may be made to the Institute Director, Professor David McAlpine (d.mcalpine@ucl.ac.uk).

Further details and instructions as to how to apply can be found on our website: www.ucl.ac.uk/ear/jobs/jobs.htm.

Closing date:7th March 2008.

UCL Taking Action For Equality.

U125175R

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

The **Proteome Centre of the University of Tübingen (PCT), Faculty of Biology,** intends to appoint an accomplished and highly motivated proteomics expert with leadership qualifications and outstanding scientific expertise in the field of

Quantitative Proteomics/Mass Spectrometry

This position as Director of the PCT at the level of a

Junior Professorship (W1, tenure-track)

will be available by 1st October 2008.

The Proteome Centre Tübingen was founded in 2003, with significant financial support of the Ministry of Science, Research and the arts, State of Baden-Württemberg, Germany. The PCT is a unit of the Interfaculty Institute for Cell Biology (IFIZ), University of Tübingen. The PCT is housed in laboratories on the Science Campus and is equipped with state-of-the-art proteomics and MS instruments. The PCT currently operates with a staff of 15 scientists, PhD students and technicians. PCT research activities focus on collaborative research projects in the fields of biology, bioinformatics, chemistry, and medicine. The new professor will direct the centre's projects and will envision new fields of research.Teaching responsibilities exist in cell biology.

The University strives to increase its proportion of female staff and specifically encourages qualified women to apply. Applications with detailed information on the applicant´s qualifications, including three named contacts for the provision of recommendations, should be submitted by no later than **20th March 2008** to

Prof. Dr. Hanspeter A. Mallot · Dean's Office · Faculty of Biology
Eberhard-Karls-Universität Tübingen
Auf der Morgenstelle 28 · 72076 Tübingen · Germany
Disabled persons with corresponding aptitude for the position will be favoured.

W124725R

UNIVERSITY OF
LIVERPOOL

Department of Chemistry

2 Postdoctoral Researchers in Polymer Synthetics

£28,289 pa

You will join the Liverpool Materials Chemistry Group and Centre for Materials Discovery (www.materialsdiscovery.com). Synthetic experience is primary to the post but additional experience in any of the following areas would also be advantageous: porous materials, gas sorption measurements, organometallics, X-ray diffraction, handling of air-sensitive materials, supercritical fluids. You should have a PhD in Chemistry or closely related discipline and an excellent publication record in the area of materials chemistry. The posts are available for 18 months initially.

Job Ref: R-567328/N **Closing Date: 7 March 2008**

For full details, or to request an application pack, visit
www.liv.ac.uk/working/job_vacancies/
Tel 0151 794 2210 (24 hr answerphone)
Please quote Job Ref in all enquiries.
COMMITTED TO DIVERSITY AND EQUALITY OF OPPORTUNITY

U125176R

UNIVERSITY OF LEEDS

Faculty of Biological Sciences
Institute of Molecular & Cellular Biology

Research Fellow (Full-time)

Kaposi's sarcoma associated herpesvirus: virus-host cell interactions

This position is available immediately for a fixed term of 36 months.

Applications are sought for the following post-doctoral post to study the regulation of lytic gene expression in Kaposi's sarcoma associated herpesvirus (KSHV) in the laboratory of Dr Adrian Whitehouse.

The post is funded by the BBSRC and will focus on the multifunctional roles of the KSHV ORF57 protein. Specifically you will investigate the role of ORF57 protein-host cell interactions and analyse how post-translational modifications of ORF57 relate to its function.

A first or upper second class degree and PhD in Molecular Biology/Virology or a related discipline, together with a strong molecular and cell biology research background and effective practical skills at the bench are essential. You will join a highly active research group studying various aspects of oncogenic herpesvirus biology.

University Grade 7 (£27,466 - £32,796 p.a.) It is likely that an appointment will be made at or below £28,289 p.a. since there are funding limitations which dictate the level at which the appointment can start.

Informal enquiries to Dr Adrian Whitehouse tel +44 (0)113 343 7096 email a.whitehouse@leeds.ac.uk

To apply online please visit http://www.leeds.ac.uk and click on jobs. Alternatively, application packs are available from Mr Alex Bateman, Faculty Staff Recruitment Office tel tel +44 (0)113 343 8040 email fbsjobs@leeds.ac.uk

Job ref 313209 Closing date 13 March 2008

We welcome applications from all sections of the community. Textphone for deaf applicants only +44 (0)113 343 4353. All information is available in alternative formats please contact +44 (0)113 343 4146.

U125188R

WORKING TOWARDS EQUALITY AND DIVERSITY www.leeds.ac.uk

INM Leibniz Institute for New Materials

The Leibniz-Community is the consortium of currently 84 Research and Service Centers. The Centers are jointly funded by federal and state governments.

The INM - Leibniz Institute for New Materials is a basic and applied research institute in transition to new scientific fields. It has currently about 180 employees and excellent research equipment and is well supported by public and third-party funding. Its present focus is on chemical nanotechnology, where the institute holds key patents and has close international links to industry. Under new leadership, this focus is now expanded to include promising novel aspects of materials science, physics and biology.

The research group *Structure formation at small scales* investigates structures that emerge in soft, condensed matter, in particular in colloids and polymers. We aim to understand their formation mechanisms, tune the results and apply them to systems of practical importance.

For this group, we seek to appoint

Post-Doctoral Researchers
Ph.D. students

who will work on projects such as the **formation of colloidal superstructures, polymer patterning for tuned adhesion,** and **nanoparticle growth mechanisms.**

Candidates should have completed their diploma, master's degree or equivalent degree in **physics, chemistry or materials science; engineers** are highly welcome as well. Post-Doctoral positions require a PhD in a related field. Experience with chemical labs, microscopy or ultramicroscopy techniques are helpful.

We can offer you a very dynamic environment with groups from biology, physics, chemistry and modelling. Mutual support, respect and open communication are highly valued. Financial compensation will be competitive and depends on previous research experience. The location of the institute is Saarbrücken, a lively town near the German-French border with fast connections into both countries. The INM promotes the occupational opportunities of female scientists, who are therefore especially encouraged to apply.

Please contact the group leader (including your CV and list of publications) if you are interested: **Dr. Tobias Kraus, INM - Leibniz Institute for New Materials, Campus D2 2, 66123 Saarbrücken, Germany, tobias.kraus@inm-gmbh.de, http://www.inm-gmbh.de**

Weitere Informationen zur Leibniz-Gemeinschaft finden Sie unter **www.leibniz-gemeinschaft.de**

Leibniz Gemeinschaft

W125062R

nature materials

Associate Editor

Nature Materials is a prestigious international monthly journal (Impact Factor 19.194) covering all aspects of materials science and technology. We have an exciting opportunity available for a materials scientist or a chemist to join our editorial team as an Associate Editor working on all aspects of the journal.

We are particularly interested in applicants with expertise in physical chemistry and soft matter research but we would welcome applications from outstanding candidates in any area of materials science.

The ideal candidate should have a PhD and preferably postdoctoral experience with a strong research record. The successful candidate will play an important role in determining the representation of their field in the journal, and will work closely with the other editors on all aspects of the editorial process, including manuscript selection, commissioning and editing of Reviews and News & Views, and writing for the journal. A key aspect of the job is liaising with the scientific community through laboratory visits and international conferences.

This is a demanding and intellectually stimulating position. Broad scientific knowledge and training, excellent literary skills and a keen interest in the practice and communication of science are a prerequisite. The successful candidate must, therefore, be dynamic and outgoing and have excellent interpersonal skills. The salary and benefits will be competitive, reflecting the critical importance and responsibilities of this position.

The new editor will join our team in our London office. The *Nature Materials* team is part of a dynamic editorial and publishing environment that also includes *Nature*, *Nature Physics* and *Nature Nanotechnology*.

Applicants should send a CV (including their class of degree and a brief account of their research and other relevant experience), a News & View style piece (600 words or less) on a recent paper from related literature, and a brief cover letter explaining their interest in the post and their salary expectations.

The closing date for applications is Monday 25th February 2007.

To apply please send your CV and covering letter, quoting reference number NPG/LON/823 to Denise Pitter at londonrecruitment@macmillan.co.uk

All candidates must demonstrate the right to live and work in the UK to be considered for the vacancy.

nature publishing group npg

IN124059R

Lecturer in Biochemical Toxicology

Pharmaceutical Science Division

A Lectureship in Biochemical Toxicology is available within the Pharmaceutical Science Division, tenable on or before May 2008. The successful applicant will be required to carry out original research, preferably but not essentially, in nanotoxicology, systems biology (drug metabolism) or metal handling diseases. S/he will also be required to contribute to the teaching of the MPharm and MSc Pharmaceutical Science degree programmes.

The successful candidate will have research expertise in toxicology, with a PhD in Toxicology, or in Biochemistry, Pharmacology, or a related discipline having a significant toxicological component.

The appointment will be made on the Lecturer A/B salary scale currently ranging from £26,094 - £39,602 per annum plus £2,323 London Allowance per annum depending on qualifications and experience.

Informal enquiries may be made by contacting Professor Frank Kelly, Head of the Pharmaceutical Science Division (frank.kelly@kcl.ac.uk), or Dr Peter Hylands, Head of Department of Pharmacy (peter.2.hylands@kcl.ac.uk). For further information about the Division please visit http://www.kcl.ac.uk/schools/biohealth/research/pharmsci/

For an application form and full job description please send an A4 SAE to Human Resources, 4th Floor Capital House, 42 Weston Street, London SE1 3QD or email hsrecruit4@kcl.ac.uk Alternatively, a full job description and application form can be downloaded by visiting www.kcl.ac.uk/jobs. Please quote reference A1/JGA/021/08-DB on all correspondence.
Closing date: 5 March 2008.

KING'S College LONDON

University of London

Equality of opportunity is College policy

U125171RM

Tenure Track Faculty Positions in Plant Biochemistry

Agricultural Biotechnology Research Center Academia Sinica, Taiwan

Applications are invited for tenure-track research fellow positions at the Assistant or Associate level (equivalent of assistant/associate professor). The successful candidates will be expected to develop a rigorous research program in the field of plant biochemistry. Areas of interest include but are not limited to: secondary metabolism, enzymology, cell wall biosynthesis and degradation, and metabolic engineering or metabolic changes in response to disease or environmental stresses in crop or medicinal plants.

The ABRC will be in a major expansion and is developing an integrated research program in enzyme biotechnology. Several new faculty hires are anticipated over the next several years, and a new research building equipped with state-of-the-art facilities will be opened in December 2008. For more information of ABRC and Academia Sinica, please visit our websites at http://abrc.sinica.edu.tw/ and http://www.sinica.edu.tw/main_e.shtml, respectively.

Qualifications: Ph.D. in Plant Biochemistry or related field, postdoctoral experience and knowledge skills in plant metabolism. We are particularly interested in applicants who are seeking a highly collaborative research environment. Applicants should submit the following materials online, at http://abrc.sinica.edu.tw/jobs/ (a) Cover letter; (b) Curriculum vitae, including publications; (c) A summary of research accomplishments; (d) Clearly focused description of future research plans; (e) PDF copies of major publications; (f) Names and contact information for three referees. Candidates should arrange three letters of recommendation to be submitted by e-mail to: abrc@gate.sinica.edu.tw or sent by regular mail to: Faculty Search Committee, Agricultural Biotechnology Research Center, Academia Sinica, No. 128, Academia Rd. Sec. 2, Nankang, Taipei 11529, Taiwan, ROC. Review of candidates will begin on March 1 and continue until the positions are filled.

JP124215R

nature

Managing Editor

Nature is the world's leading weekly scientific journal and is the flagship publication of Nature Publishing Group.

With its authoritative journalism and opinion, a leading position in its science research content, and worldwide influence and engagement, *Nature* stands ready to undertake a period of further investment in both print and online formats. The publisher and the Editor-in-Chief of *Nature* wish to employ a senior manager who will take direct responsibility for the implementation of the publishing programme and for key aspects of publishing and editorial management.

Applicants must have a demonstrable familiarity with the scientific landscape, strong commercial drive, and the ability to manage projects and to achieve demanding goals in a way that stimulates and inspires the colleagues on whom they depend.

The job is based in the London offices of the Nature Publishing Group (NPG), and involves close interactions with colleagues in other parts of Europe, the United States and the Asia-Pacific. The Managing Editor will report to the Editor-in-Chief of *Nature* and to the Managing Director of NPG.

Candidates should have a strong commercial drive, prior editorial experience, preferably in scientific publishing, and some previous experience of the commercial side. They should be comfortable with print and online media and have experience of running projects and managing teams.

The position will be full-time.

The position is based in our modern London offices.

Please send your CV, a summary of relevant experience, and your current salary, quoting reference number to NPG/LON/815, to Geetika Juneja Personnel Assistant at londonpersonnel@macmillan.co.uk

All candidates must demonstrate the right to live and work in the UK to be considered for the vacancy.

Closing Date: 14th February 2008

nature publishing group npg

IN123677R

CARL VON OSSIETZKY universität OLDENBURG

W124828R

The Institute of Biology and Environmental Sciences at the Faculty of Mathematics and Natural Sciences invites applications for a tenured faculty position to further develop hearing sciences within the Lower Saxonian „Centre for Hearing Research". The following position can be filled starting from 1 May 2008:

Professor of Cochlea/ Brainstem Physiology (W2)

We seek outstanding candidates with an established research program in auditory physiology on the cellular or systemic level. The successful applicant will have an international reputation and a record of external funding. We are particularly interested in applicants focusing on the mammalian auditory system. Research participation within the DFG-funded transregional collaborative research centre SFB/TRR 31 „The Active Auditory System" and contributions to the International Graduate School „Neurosensory Science, Systems and Applications" are desired. The successful applicant will teach courses in the BSc and MSc program in „biology", in the MSc program „Audiology and Hearing Technology" and in the PhD program in „Neurosensory Science and Systems". A reduced teaching load until the end of 2012 allows developing a strong research program.
Qualifications are specified in §25 NHG.
Since the Universities of Bremen and Oldenburg have concluded a cooperation agreement, active contributions to this cooperation are desirable.
The Carl von Ossietzky University of Oldenburg is an equal opportunity/affirmative action employer. In order to increase the percentage of female faculty members, female candidates with equal qualification will be given preference. Applicants with disabilities will be preferentially considered in case of equal qualification.

Applications including the usual documents (curriculum vitae, list of publications, certificates, diplomas, and list of previous teaching activities) should be sent by **16 March 2008** to the **Carl von Ossietzky Universität, Director of the Institute of Biology and Environmental Sciences, Faculty V, D-26111 Oldenburg.**

Ph.D.-scholarship

A PhD scholarship is available in the Laboratory of Neurobiology, Faculty of Engineering, Science and Medicine at Aalborg University and Institute of Human Genetics at Aarhus University (Denmark).

The successful candidate will join a research group consisting of medical doctors, biochemists, biologists, medical students and technicians, working on treatment of diseases in the central nervous system (CNS) with a special focus on neurobiology. The group's main focus is a design of nanocarriers for gene therapy of neurological disorders. The work is performed using both in vitro and in vivo experiments in cell cultures and animal models. The work is done in a stimulating international environment and in a collaboration with research groups from other countries.

The successful candidate is expected to work at the Laboratory of Neurobiology, Aalborg University as well as at the Institute of Human Genetics, Aarhus University.

Applicants should possess a M.Sc. or M.D. degree and have an interest in pharmacology, among others in preparation of conjugates e.g. liposomes, which will allow drug delivery into a brain through the blood-brain barrier. Other tasks will include: establishing and maintenance of a primary culture of brain endothelial cells and other cell cultures, working with plasmid DNA and thereto related molecular biology techniques. The candidate's personal scientific interests and ideas will be taken into account during the course of the project.

For further information please contact: Professor, dr.med., ph.d. Torben Moos, +45 99402420, tmoos@hst.aau.dk and Professor, dr.med, MD Thomas G. Jensen, +45 8942 1677, thomas@humgen.au.dk

The candidates' qualifications will be evaluated by an appointed evaluation comitee consisting of specialists in the field. Based on the evaluation the Dean of The Faculty of Engineering, Science and Medicine will grant the stipend.

The commencement date of the scholarship is on 1.4.2008 or soon after, ph.d.-stip.nr. 562/06-nr.

PhD scholarship duration is 3 years. Every PhD student becomes automatically a member of The International Doctoral School of Technology and Science, which predicts a further research education according to a declaration nr. nr. 114, dated 8. March 2002 about the PhD degree. According to the declaration, research progress and education is evaluated every 6-months. A positive evaluation is a condition for further payment of the stipend.

Employment and herewith offered competitive salary will be according to the agreement about employment and salaries for academic personnel in Denmark, the Ministry of Finance from the 11.01.2006 and the 13.06.2007.

Questions concerning employment and salary can be directed to Dorte Antonsen, Office of The Faculty of Engineering, Science and Medicine, e-mail: doa@adm.aau.dk, tel. +45 9635 7380.

The application from can be downloaded from: http://adm.aau.dk/fak-tekn/phd/vacant/index.htm

The Faculty of Engineering, Science and Medicine has The International Doctoral School of Technology and Science: http://adm.aau.dk/fak-tekn/phd/index.htm and a ph.d.-association (PAU) for all AAU PhD students: http://www.pau.aau.dk

Applications labelled "ph.d.-stip.nr. 562/06-nr.", master diploma, CV and other relevant information should be sent in 4 copies and should arrive at Aalborg University not later than on the 3.3.2008 before noon.

Electronic applications will not be considered.

Applications should be sent to:
Aalborg Universitet, De Ingeniør-, Natur- og Sundhedsvidenskabelige Fakulteter, Niels Jernes Vej 10, 9220 Aalborg Øst, Denmark

W125160R

Imperial College London

Division of Molecular Biosciences
Faculty of Natural Sciences

RCUK Fellowship in Biopharmaceutical Processing

Salary: £38,880 - £43,420 per annum

Imperial College is ranked the fifth best university in the world (Times Higher QS World University Rankings 2007).

We are seeking to appoint an RCUK fellow in the Division of Molecular Biosciences within the Department of Life Sciences, Imperial College London. The fellowship is part of a joint initiative between the Division and the Department of Chemical Engineering in the area of Biopharmaceutical Processing building upon exceptional research strengths in both groupings. The emphasis of the research area pertaining to this position is in the application of molecular and systems biology approaches primarily to look at proteomic and transcriptomic applications in the production and downstream processing of biopharmaceuticals. Candidates with a strong biological background who are interested in moving into the area are also encouraged to apply. The RCUK Fellowship will support the post for the first five years and it is expected to lead to an open-ended academic post.

Information about the RCUK Fellowship scheme, including eligibility criteria, is available at http://www.rcuk.ac.uk/acfellow/ Candidates should note that this scheme is only open to researchers supported on any type of fixed-term grant funding and to holders of current fellowship awards or other researchers without current research support.

The Division is housed in state-of-the-art laboratories on the South Kensington Campus. Further information about the Division can be found at the following website: http://www.imperial.ac.uk/molecularbiosciences You will also be expected to fully participate in the teaching and administrative activities of the Division. The fellow will also work closely with colleagues in Bioprocessing in the Department of Chemical Engineering and more information can be found at the following website http://www3.imperial.ac.uk/chemicalengineering
To make informal enquiries about the post please contact Professor Paul Freemont by e-mail: p.freemont@imperial.ac.uk.

An application form, further particulars including a job description and person specification can be obtained from the following links:
http://www3.imperial.ac.uk/employment/research

Completed application forms should be emailed to c.simmons@imperial.ac.uk or posted to
Ms Coralie Simmons, Division of Molecular Biosciences, Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, Flowers Building, South Kensington, London SW7 2AZ.

Closing date: 2 April 2008 (12 noon).

U125217R

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Your creativity, our technology, fighting together against infectious disease

tibotec

BELUGA

Sr. Scientist - HIV Virology

Mechelen/Belgium Ref. 0800573

Tibotec is a pharmaceutical research and development company with the mission to be a world leader in the discovery and development of innovative HIV/AIDS drugs and superior anti-infectives for diseases of high unmet medical need. Recognised as one of the companies at the forefront of HIV research, Tibotec has 3 HIV compounds in clinical development, and a number of research/early development programs in HIV, HCV and TB. Tibotec is committed to improving patient care and quality-of-life.

We currently have positions for (m/f):

Your job:

- You will work within a research team and co-operate closely with other teams, which focus on specific protein targets, to create novel assays to investigate those targets. The focus will be on assay systems to find innovative small-molecule drugs against HIV.
- Your function: Senior Scientist in HIV Target & Drug Discovery - PhD level.

Your profile:

- You will have considerable knowledge in the field of virology and infectious diseases.
- You should have a PhD degree in biology or a related field, or at least three years of practical experience in protein biochemistry in particular enzyme kinetics, protein purification/recovery and peptide handling. Knowledge with SPR technology and Molecular Biology would be desirable.
- You should be able to independently and proactively apply this knowledge in the design and execution of experimental work, to enable progression of assay development projects.
- You are expected to act as a resource in applying your technical and experimental knowledge to the work of other scientists in the departments and other therapeutic areas.
- This position calls for a creative personality with the ability to interact efficiently with researchers in different fields and a strong drive towards scientific innovation.
- Good communication skills are required.

Sr. Scientist - HCV Virology

Mechelen/Belgium Ref. 0800495

Your job:

- Role is key in rapidly building up our expertise in the genetic and functional characterization of clinical and laboratory strains of HCV exhibiting virological breakthrough (ie resistance). Involves developing and connecting the laboratory cellular & biochemical assay platforms to the needs of the Compound Development and Discovery Teams for the Tibotec HCV programs.
- Your function: Senior Scientist in HCV Target & Drug Discovery - PhD level.

Your profile:

- PhD in Biological Sciences or Pharmacy + at least 2 years relevant postdoctoral experience in academia or industry.
- Experience in the principles of classical RNA virus virology, preferably with expertise in molecular cloning and qPCR technology, nucleic acid and protein sequence analysis, cellular pathway analysis, and antiviral screening approaches. Proven expertise in scientific writing and communication.
- You will participate in multidisciplinary project teams involving medicinal chemistry and immunology and play an important role in external drug discovery collaborations

You will be expected to interpret data and generate reports on data generated from incoming clinical serum samples from clinical trials and report back to the respective Clinical Virology members of each Compound Development Team

Successful candidates will enjoy a competitive salary as well as advancement opportunities in a young, dynamic group. If you are interested in the above mentioned positions please submit your application in STARs on www.tibotec.com or to:

Tibotec Bvba, to Tine Van den Brande, Intercity Business Park, Generaal De Wittelaan L11, B3
2800 Mechelen - tvdbran2@tibbe.jnj.com - fax: +32 15 286 347

www.tibotec.com

W125231R

Loughborough University

Human Sciences

Lecturer/Senior Lecturer in Psychology (3 posts)

Minimum £30,012 per annum

Applications are invited for three psychology lectureships, with one of these earmarked for appointment at senior lecturer level. Applicants from across the field of psychology are encouraged to apply, although priority will be given to candidates with research interests that build upon our existing strengths in ageing; cognitive performance and disability; nutrition; exercise and physical activity; sleep and insomnia; work, health and wellbeing; and reactions and performance in extreme environments. Appointees will be expected to contribute towards teaching at undergraduate and postgraduate levels and demonstrate a strong commitment to maintaining the Department's excellent standards in learning and teaching.

Please indicate clearly on your application form which position you are interested in by quoting the relevant reference number(s) which can be found on the job descriptions for each post.

Informal enquiries to Professor Roger Haslam, Head of Department, email: R.A.Haslam@lboro.ac.uk, tel: 01509 223042.

Closing date: 7 March 2008. Curriculum Vitae will only be accepted if accompanied by a completed University application form.

U124017R

www.lboro.ac.uk/jobs

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French Institute of Health and Medical Research

Atelier de formation 185
Intrinsically disordered proteins and associated pathologies: prediction, characterization and function

Organizers: Richard Haser (IBCP, Lyon), Sonia Longhi (AFMB, Marseille)

● **Phase I**
Critical assessment…: May 19-20, 2008 ● Saint-Raphaël

Aims
In recent years it was established that eukaryotic genomes code for a high proportion of intrinsically disordered proteins (IDPs). IDPs are functional despite lack of stable II and III structures under physiological conditions. They are often involved in cellular regulation and signalisation processes. Structural disorder is also found in a number of proteins implicated in severe neurodegenerative diseases (Alzheimer, Parkinson…). The main goal of this workshop is to familiarize participants with structural disorder and induced folding. This workshop also aims at providing present knowledge of the role of these proteins, their propensity to fold, the molecular mechanisms that control folding and unfolding, and the impact of these latter phenomena in terms of functional implications and of relationships with various pathologies.

Audience
Researchers in molecular and cellular biology, post-doctoral fellows and lecturers, engineers, PhD students and post-docs involved in the fields of molecular biology, biophysics, biomedecine and structural biology.

Lectures will be given in English.

Programme
A first part will focus on the features and roles of IDPs, and on bioinformatics approaches for predicting structural disorder. Then, the experimental techniques and methods to assess structural disorder and induced folding will be presented through well-characterized IDPs. Finally, the relationships between protein disorder and associated pathologies will be discussed in light of present therapeutical approaches for the treatment of neurodegenerative diseases.

● **Phase II**
Technical workshop: 23-25 June 2008 ● Lyon

Programme
The first part of this practical course will focus on the use of various bioinformatics tools for the prediction of disorder. The second part will be devoted to the use of techniques such as circular dichroism, fluorescence spectroscopy, dynamic light scattering, NMR (liquid, solid) and small angle X-ray scattering (SAXS), for the experimental assessment of structural disorder.

Selection
10 participants will be selected among participants to phase I.

With the participation of : Martin Blackledge (Grenoble, France), Keith Dunker (Indianapolis, USA), David Eliezer (New York, USA), Patrice Gouet (Lyon, France), Sonia Longhi (Marseille, France), Richard Kriwacki (Memphis, USA), Monika Fuxreiter (Budapest, Hungary), Guy Lippens (Lille, France), Anne Poupon (Orsay, France), Véronique Receveur-Bréchot (Marseille, France), Peter Tompa (Budapest, Hungary), Vladimir Uversky (Indianapolis, USA), Peter Wright (San Diego, USA).

Registration deadline: March 14, 2008

W123505A

University of Glasgow

Faculty of Medicine – Division of Clinical Neuroscience

Research Assistant

£29,139 – £32,796

Olfactory ensheathing cells (OECs) and olfactory stem cells (OSCs) are promising cells for transplant-mediate repair strategies of CNS injury. The aim of this project funded by the CSO for 2.5 years is to define the culture conditions to promote the growth and differentiation of both human OECs and human OSCs and test their reparative capacity in spinal cord injury models.

Applications (CV) plus email address of referees should be submitted to Prof Sue Barnett, Division of Clinical Neuroscience, Beatson Labs, Garscube Estate, Switchback Road, Glasgow G61 1BD, email: s.barnett@beatson.gla.ac.uk quoting Ref 14054/DPO/A3. Closing date: 14 March 2008

Institute of Biomedical & Life Sciences
Division of Infection & Immunity

Research Assistant

£29,139 – £32,796

The University of Glasgow is renowned for its excellence in Molecular Parasitology and the infrastructure of the laboratories comprises state of the art facilities. This Wellcome Trust-funded project in the laboratory of S. Müller (s.muller@bio.gla.ac.uk, www.gla.ac.uk:443/ibls/staff/staff.php?who=PQS|~~) within the Wellcome Centre for Molecular Parasitology will investigate metabolic networks of the malaria parasite Plasmodium falciparum. You will employ molecular biology, biochemistry, reverse genetics, cell biology and metabolic labelling to dissect the precise role of key players in the parasite's metabolism. You will be a highly motivated and enthusiastic scientist and will have extensive experience in the techniques required for this position. This post is available for up to three years. www.gla.ac.uk/jobs/vacancies/researchandteaching/14049ra/#d.en.64546

Applications should be sent to FBLS Staffing Office, West Medical Building, University of Glasgow, Glasgow, G12 8QQ, UK quoting Ref 14049/DPO/A3 (re-advertisement of 13783).

Research Assistant

£29,139 – £32,796

This MRC-funded position in the laboratory of J C Mottram (j.mottram@bio.gla.ac.uk, www.gla.ac.uk/centres/wcmp/research/mottram) is available to carry out a post-genomic study of peptidases in the parasitic protozoon Leishmania within the Wellcome Centre for Molecular Parasitology. The project will investigate the role of peptidases in differentiation and virulence of Leishmania. You should have strong experience in molecular biology, biochemistry and/or cell biology. A background in parasitology would be an advantage but is not essential. You will join a team of postdoctoral research assistants and postgraduate students engaged on studies on drug targets in parasitic protozoa. The post is available for up to five years. www.gla.ac.uk/jobs/vacancies/researchandteaching/14050ra/#d.en.64550

Applications should be sent to FBLS Staffing Office, West Medical Building, University of Glasgow, Glasgow, G12 8QQ, UK quoting Ref 14050/DPO/A3 (re-advertisement of 13864).

Closing date for the above two posts: 29 February 2008.

Previous applicants need not apply. Further particulars can be found at www.glasgow.ac.uk/jobs/vacancies/

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U125262RM

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IN125042EL

The champagne award

Planning for the future.

Gregory Benford

The first case of the morning was typical.

Roger scanned the cyberforms of a woman in her forties, applying for a child using her entire KidCred. No father to be named, no mention of how she would get pregnant. A clinic, he guessed, but maybe she had a donor. Not the Reproduction Office's business, no. He offered her the government Champagne Award, good for a reasonable bottle, but she didn't take it.

Next came a rather different case. Roger eyed the late-20s woman and two obviously gay men in his office. He made the usual ritual greeting, outlined the documents they needed, and they produced them, all filled out in her crisp hand. She would be the surrogate for the two men, who were each putting up half their KidCred. What they paid her was a private matter. She would have no claim on the child. She took the Champagne Award, though.

That job went quickly and then came something awful. A fretful couple came in, breathing too quickly and nervous.

"Our child died the second day in the hospital," the husband explained.

His wife blurted: "A blood disorder, they're still trying to figure it out."

Before Roger could say a word the husband turned furious. "But they say there's nothing they can do! It's their fault!"

Roger knew enough to study the hospital readouts first. The child had lasted 36.7 hours and then died of some sort of haemorrhage. The couple were in their forties and had been warned of the risks, some of them genetic.

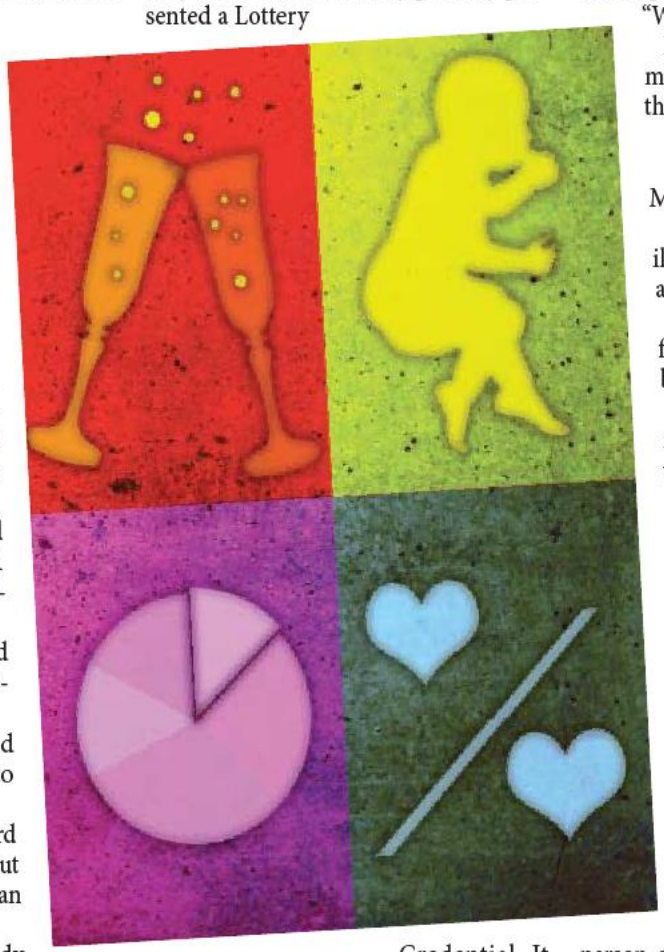
He was no doctor, but he knew the rules. "It's beyond 24 hours," he said calmly, voice grey and dead. "Carried to full-term, no intervening circumstances. You cannot get reversed credit."

It took an hour to get them out of the office. Roger nearly called the guards. The couple wouldn't talk to the counsellors because they knew that was just a bureaucratic dodge. But there was really nothing he could do. Rules were rules.

His long-term girlfriend Lucy called but he was too busy to talk; paperwork.

After his coffee break there was a lesbian couple, duly married, each using her last half KidCred. Nothing unusual, documents in order, and they took the Champagne Award, too.

The next couple was a man and wife bubbling over with joy. Roger liked to see that. The couple already had the max, two healthy children, but wanted a third; they were Catholic. They proudly presented a Lottery Credential. It looked standard. The winning number was in the right code. Roger shared in their celebration while he ran it through the verifier — which went bing.



JACEY

Everybody froze. They didn't know what it meant, but Roger did. He had to explain to them that it was a very well made fake. The original, authentic number had been used two weeks before. That was how the counterfeiters had obtained the publicly posted number; the rest was technology.

This time he had to call the guards. The husband kept shouting "I paid three hundred thousand in tickets!" over and over, but the tickets he had were fake, too.

Pretty depressing, but there were rules. Every game had them. To win the game you had to play.

Lucy called; he was busy filing reports. Tough day; he took an early lunch. In the basement cafeteria he sat with Henry from Document Authentication and Mary from Statistics. They were partners, not married but had lived together for two decades. And today they were quitting, they announced happily.

"We played it just right," Henry said, although he didn't seem all that elated. "Waited for the market peak."

Mary was joyous. "I got nearly a million! Think what we can do with that."

"Who from?" Roger asked.

"A couple that wanted a third." Mary rolled her eyes. "Filthy rich."

Roger blinked. "The fines for illegal birth are nearly that much anyway."

Henry said: "Plus no education for the no-KidCred child, no social benefits …"

Mary said firmly: "I predict no-cred parents will get prison time in a few more years."

Henry looked down at his plate. "If we invest this the right way, we can retire and manage our stock."

"You might get paid even more later," Roger said. "There are rumours that the KidCred standards may get tougher."

"Those rumours are why the market has run up. We're just cashing in, like the gays." Mary merrily toasted with her water glass. "Here's to cap and trade!"

Henry nodded, although he looked sad. "Roger, I can't see them taking away the one person, one child rule, though." He looked down.

Every game had its winners and losers, Roger thought. Gay guys had little interest in children, so they won big. With 11.6 billion souls in the world, what else could humanity do? Prison for unlicensed childbearing didn't seem implausible to him at all.

But to win the game, you had to play.

So when his telephone rang, back at his desk, he was delighted to hear from Lucy. Somehow the day crystallized for him. Without thinking, he said suddenly: "How would you like a bottle of champagne?" ■

Gregory Benford is professor of physics at the University of California, Irvine, and a novelist.

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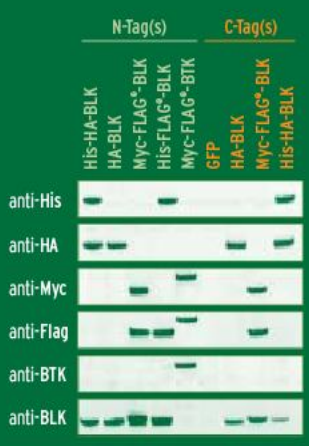

The Western blot analysis of HEK293 cell lysate over-expressing BLK or BTK tagged with indicated epitopes.

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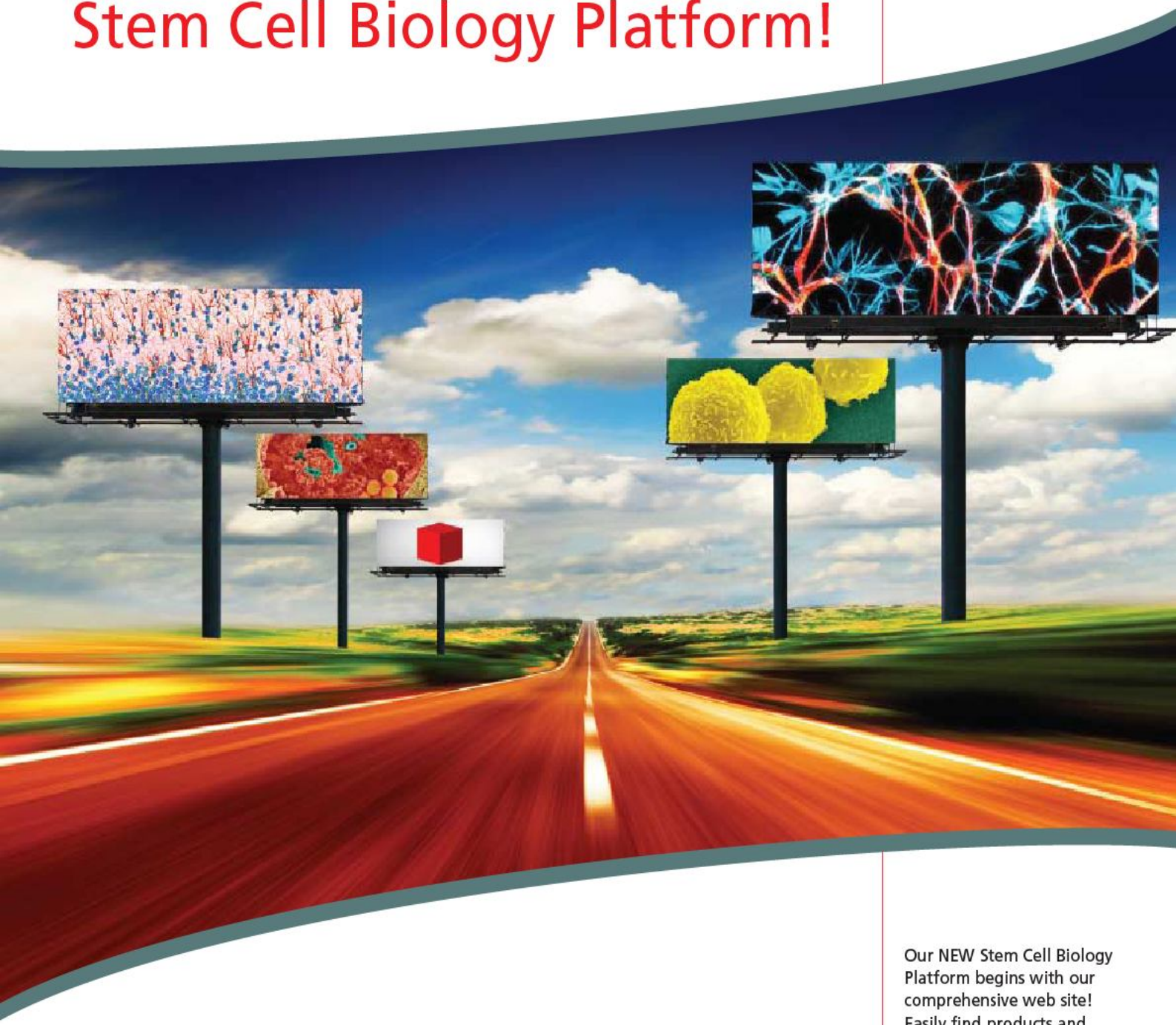

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In addition to our product profiles you will also find the latest references, application notes, and a new Bulletin Board feature that will enable stem cell scientists like you to consult and exchange ideas and information!

Our Innovation, Your Research — Shaping the Future of Life Science

Our NEW Stem Cell Biology Platform begins with our comprehensive web site! Easily find products and detailed usage information in the following stem cell areas:

- Isolation
- Expansion
- Characterization
- Differentiation
- Functional Profiling
- In-vivo tracking and
- Much More!